

Highly Potent 2-Oxoester Inhibitors of Cytosolic Phospholipase A₂ (GIVA cPLA₂)

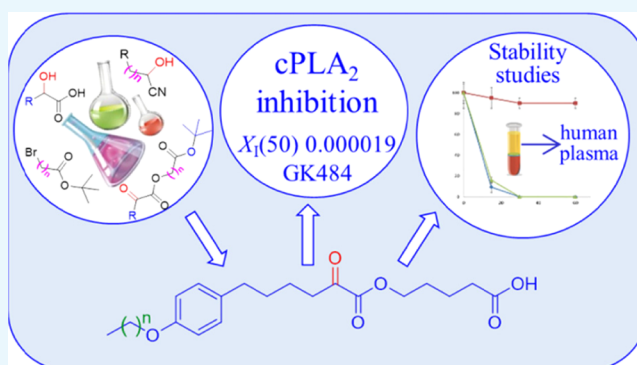
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S Supporting Information

ABSTRACT: Cytosolic phospholipase A₂ (GIVA cPLA₂) 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 cPLA₂ presenting X₁(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 A₂ (PLA₂s) 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 PLA₂ (GIVA cPLA₂) 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 PLA₂ have been recently summarized by Leslie.⁴ Due to the involvement of PLA₂s 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 PLA₂ 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 PLA₂ 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 cPLA₂ by inhibitor **2** (Figure 1) blocked *Streptococcus pneumoniae*-induced polymorphonuclear cells transendothelial 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 CCl₄, indicating that a GIVA cPLA₂ 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 cPLA₂ 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 cPLA₂ 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 cPLA₂ inhibitors highlight the importance of identifying new highly potent inhibitors to regulate the activity of GIVA cPLA₂. Last year, we reported the development of a novel class of GIVA cPLA₂ 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 cPLA₂ in vitro inhibition (X₁(50) 0.000078). The aim of the present work was to further understand the characteristics of 2-

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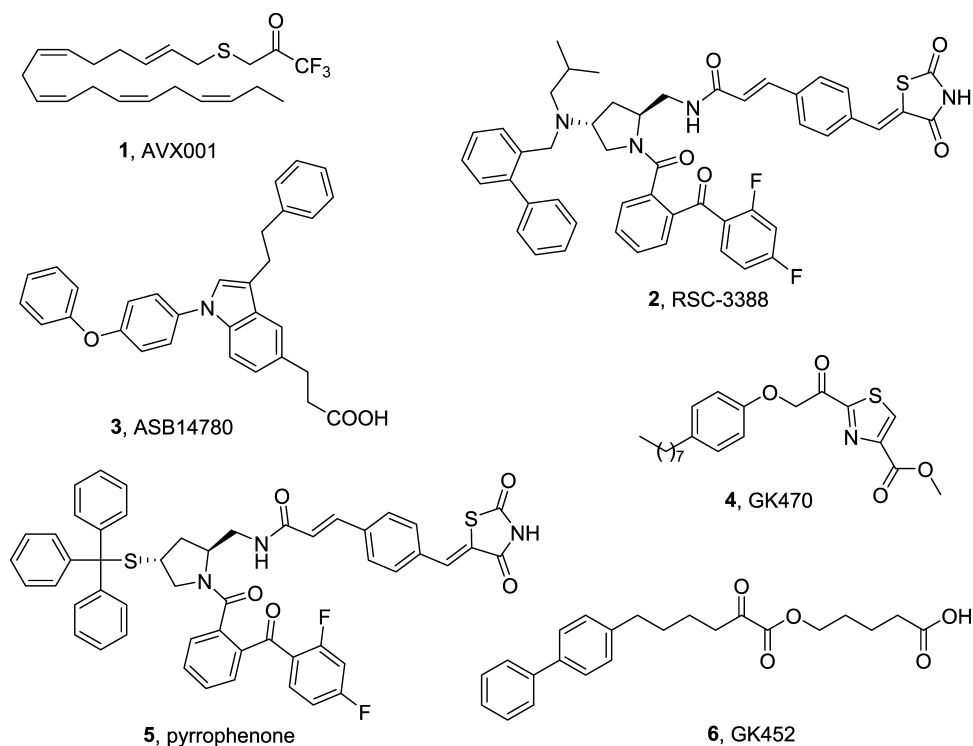
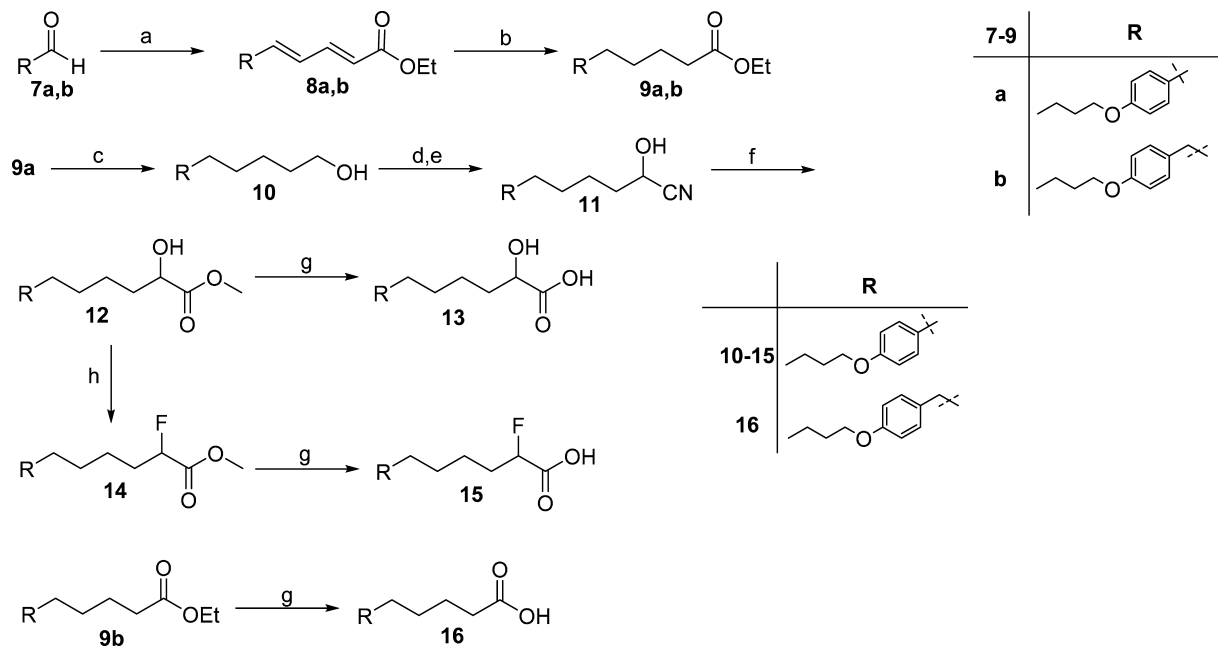


Figure 1. Structures of known GIVA cPLA₂ inhibitors.

Scheme 1



^aReagents and conditions: (a) $(\text{C}_2\text{H}_5\text{O})_2\text{P}(\text{O})\text{CH}_2\text{CH}=\text{CHCO}_2\text{C}_2\text{H}_5$, $\text{LiOH}\cdot\text{H}_2\text{O}$, tetrahydrofuran (THF), mol. sieves; (b) H_2 , 10% Pd/C, EtOH; (c) DIBALH, Et_2O ; (d) $\text{C}_6\text{H}_5\text{I}(\text{O}_2\text{CCH}_3)_2$, 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 .

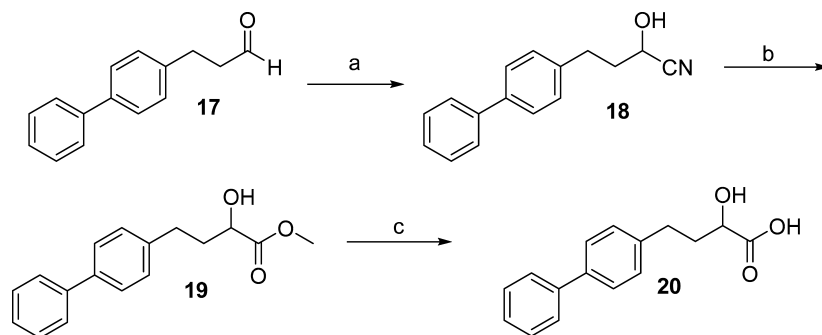
oxoesters and explore the possibility of producing more potent GIVA cPLA₂ inhibitors.

RESULTS AND DISCUSSION

Synthesis of Inhibitors. To extend the structure–activity relationship studies on 2-oxoester inhibitors, we (a) shortened

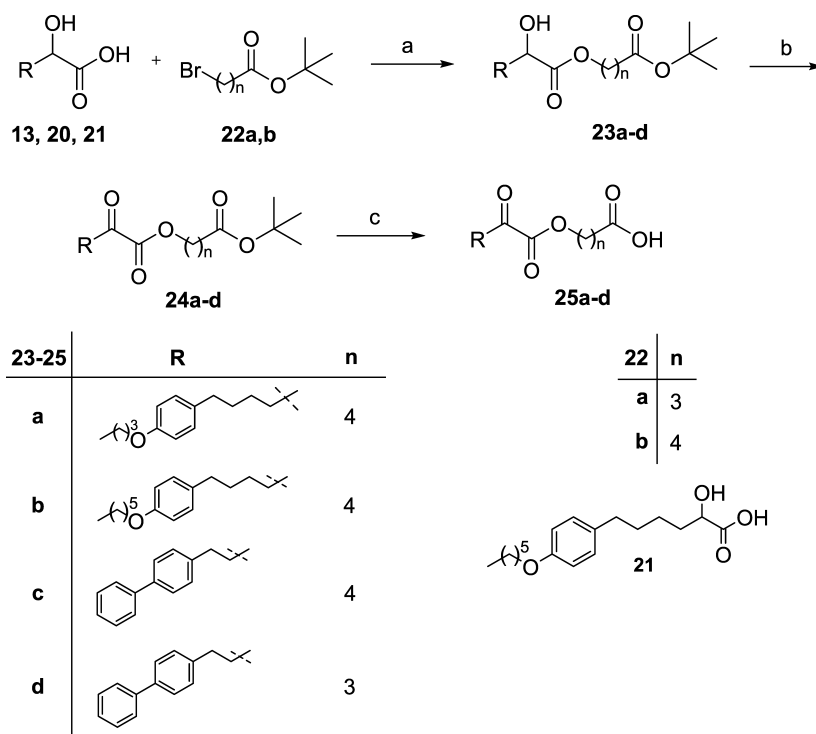
the length between the activated carbonyl group and the biphenyl functionality, (b) replaced the biphenyl system with a *para*-alkoxy-substituted phenyl group, and (c) explored the importance of the activated carbonyl group. To this end, a variety of 2-substituted carboxylic acids, required for the target compounds, were synthesized as described in Scheme 1.

Scheme 2



^aReagents and conditions: (a) (i) 4 N aq. NaHSO₃, CH₂Cl₂, and (ii) 4 N aq. KCN; (b) 4 N HCl/MeOH; and (c) 50% aq. NaOH, 1,4-dioxane.

Scheme 3



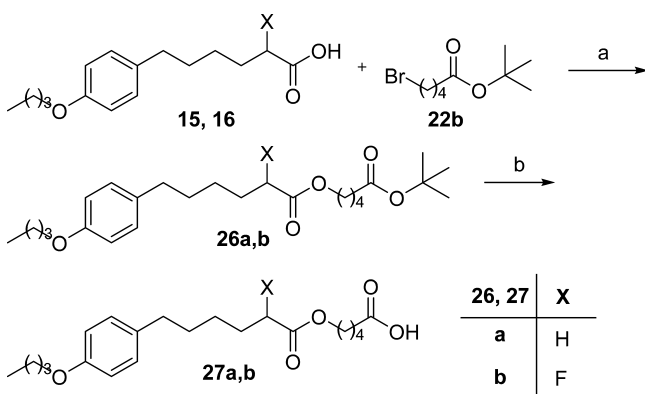
^aReagents and conditions: (a) 20% aq. Cs₂CO₃, *N,N*-dimethylformamide (DMF); (b) Dess–Martin periodinane, CH₂Cl₂; and (c) 50% trifluoroacetic acid (TFA)/CH₂Cl₂.

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).

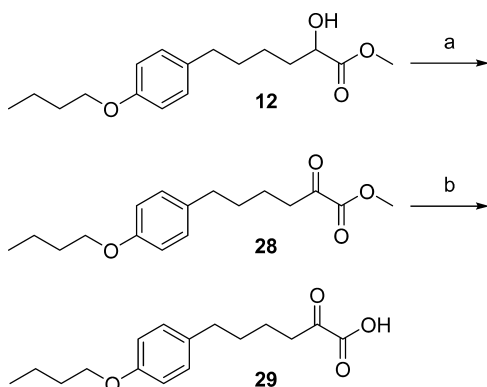
Likewise, reaction of 2-fluoro carboxylic acid **15** and acid **16** with *tert*-butyl bromopentanoate (**22b**) resulted in derivatives **26a,b**, and subsequently, after the removal of *tert*-butyl group, to the desired products **27a,b** (Scheme 4). Finally, the synthesis of 2-oxoacid **29** was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

Scheme 4



^aReagents and conditions: (a) 20% aq. Cs₂CO₃, DMF and (b) 50% TFA/CH₂Cl₂.

Scheme 5



^aReagents and conditions: (a) Dess–Martin periodinane and (b) 20% aq. Cs₂CO₃, MeOH.

In Vitro Inhibition of GIVA cPLA₂, GVIA iPLA₂, and GV sPLA₂. All of the new compounds synthesized were tested for in vitro inhibition of human GIVA cPLA₂, calcium-independent phospholipase A₂ (GVIA iPLA₂), and secreted phospholipase A₂ (GV sPLA₂) 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 PLA₂ 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 cPLA₂ 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 cPLA₂ 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 results¹⁴ 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 iPLA₂ 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 cPLA₂ was observed. Compound **25b** (GK484) is the most potent inhibitor of GIVA cPLA₂ 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 iPLA₂ (86 and 77%, respectively, at a high mole fraction 0.091). In our experience, compounds that show inhibition of PLA₂ 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 cPLA₂ inhibitor **25b** with a X_I(50) 0.000019 is the most potent inhibitor of GIVA cPLA₂ ever reported and is at least 500-fold selective over GVIA iPLA₂.

To confirm the importance of the oxoester functionality, we removed the activated carbonyl group. Compound **27a** (GK505) abolished the inhibitory potency against GIVA cPLA₂. 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 cPLA₂ 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 cPLA₂ was observed. Finally, 2-oxoacid **29** (GK511) was evaluated in vitro and only a very weak inhibition of GIVA cPLA₂ 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 cPLA₂ 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/*

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

No (Code)	Structure	ClogP ^a	GIVA cPLA ₂		GVIA iPLA ₂		GV sPLA ₂
			% Inhibition ^b	X _i (50)	% Inhibition ^b	X _i (50)	% 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

^aCalculated 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 C₅H₈O₂ (−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

cPLA₂ 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 cPLA₂. 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. ¹H, ¹³C, and ¹⁹F NMR spectra were recorded on Varian Mercury at 200, 50, and 188 MHz, respectively. CDCl₃ 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.

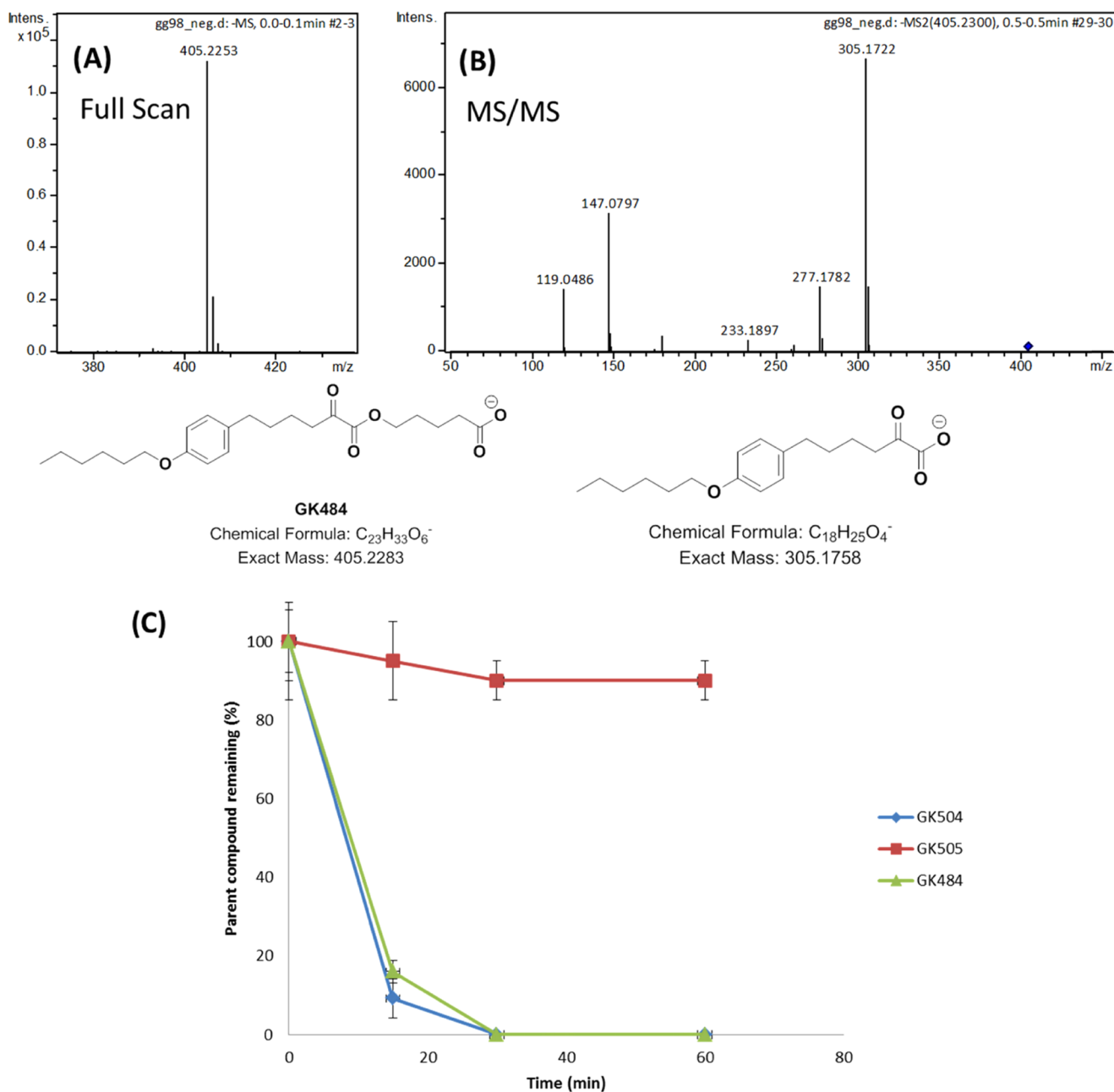


Figure 2. Precursor ion (A) and MS/MS HRMS spectrum (B) of inhibitor GK484. Time-dependent degradation of inhibitors GK484, GK504, and GK505 in human plasma (C).

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·H₂O (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; ¹H NMR (200 MHz, CDCl₃): 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, CH₂OCO), 3.87 (t, *J* = 6.4 Hz, 2H, CH₂O), 1.82–1.60 (m, 2H, CH₂), 1.47–1.33 (m, 2H, CH₂), 1.25 (t, *J* = 7.1 Hz, 3H, CH₃CH₂OCO), 0.92 (t, *J* = 7.3 Hz, 3H, CH₃); ¹³C NMR (50 MHz, CDCl₃) 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 C₁₇H₂₂O₃: 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 ¹H NMR (200 MHz, CDCl₃): 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,

2x=CH), 5.79 (d, $J = 15.3$ Hz, 1H, =CH), 4.16 (q, $J = 7.1$ Hz, 2H, CH₂OCO), 3.89 (t, $J = 6.3$ Hz, 2H, CH₂O), 3.59–3.30 (m, 2H, PhCH₂), 1.81–1.63 (m, 2H, CH₂), 1.45 (m, 2H, CH₂), 1.25 (t, $J = 7.1$ Hz, 3H, CH₃CH₂OCO), 0.96 (t, $J = 7.3$ Hz, 3H, CH₃); ¹³C NMR (50 MHz, CDCl₃): 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 C₁₈H₂₄O₃: 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 H₂ 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; ¹H NMR (200 MHz, CDCl₃): 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, CH₂OCO), 3.92 (t, $J = 6.4$ Hz, 1H, CH₂O), 2.56 (t, $J = 6.9$ Hz, 2H, PhCH₂), 2.31 (t, $J = 7.0$ Hz, 2H, CH₂CO), 1.84–1.70 (m, 2H, CH₂), 1.69–1.56 (m, 4H, CH₂), 1.56–1.38 (m, 2H, CH₂), 1.24 (t, $J = 7.1$ Hz, 3H, CH₃CH₂O), 0.97 (t, $J = 7.3$ Hz, 3H, CH₃); ¹³C NMR (50 MHz, CDCl₃): 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 C₁₇H₂₆O₃: C, 73.35; H, 9.41; found: C, 73.07; H, 9.69.

Ethyl 6-(4-Butoxyphenyl)hexanoate (9b). Yield 99%; colorless oil; ¹H NMR (200 MHz, CDCl₃): 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, CH₂OCO), 3.87 (t, $J = 6.4$ Hz, 2H, CH₂O), 2.64–2.39 (m, 2H, PhCH₂), 2.33–2.18 (m, 2H, CH₂CO), 1.86–1.30 (m, 10H, 5 × CH₂), 1.22 (t, $J = 7.2$ Hz, 3H, CH₃CH₂O), 0.94 (t, $J = 7.2$ Hz, 3H, CH₃); ¹³C NMR (50 MHz, CDCl₃): 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 C₁₈H₂₈O₃: 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 Et₂O (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; ¹H NMR (200 MHz, CDCl₃): 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, CH₂O), 3.60 (t, $J = 6.5$ Hz, 2H, CH₂OH), 2.50 (t, $J = 6.4$ Hz, 2H, PhCH₂), 2.08 (brs, 1H, OH), 1.86–1.30 (m, 10H), 0.97 (t, $J = 7.3$ Hz, 3H, CH₃); ¹³C NMR (50 MHz, CDCl₃): 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 C₁₅H₂₄O₂: 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 CH₂Cl₂ (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 Na₂S₂O₃ (10 mL), 10% aqueous NaHCO₃ (10 mL), and brine (10 mL), dried over Na₂SO₄, and concentrated in vacuo. The aldehyde was then dissolved in CH₂Cl₂ (1.5 mL) and 4 N aqueous NaHSO₃ (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 H₂O (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, H₂O (10 mL) was added and extracted with CH₂Cl₂ (3 × 10 mL). The combined organic layers were washed with brine, dried over Na₂SO₄, 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; ¹H NMR (200 MHz, CDCl₃): 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, CH₂O), 2.58 (t, $J = 7.1$ Hz, 2H, PhCH₂), 1.93–1.38 (m, 10H, 5 × CH₂), 0.98 (t, $J = 7.3$ Hz, 3H, CH₃); ¹³C NMR (50 MHz, CDCl₃): 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 + NH₄]⁺, 100); Anal. Calcd for C₁₆H₂₃NO₂: 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 CH₂Cl₂ (1.5 mL), an aqueous solution of NaHSO₃ 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 H₂O (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 CH₂Cl₂ (2 × 20 mL), washed with brine, and dried over Na₂SO₄. 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; ¹H NMR (200 MHz, CDCl₃): 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, PhCH₂), 2.22–2.13 (m, 2H, CH₂). ¹³C NMR (50 MHz, CDCl₃): 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 + NH₄]⁺, 100); Anal. Calcd for C₁₆H₁₅NO: 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 Et₂O (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; ¹H NMR (200 MHz, CDCl₃): 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, CH₂O), 3.77 (s, 3H, OCH₃), 2.83 (bs, 1H, OH), 2.63–2.48 (m, 2H, PhCH₂), 1.93–1.37 (m, 10H, CH₂), 0.97 (t, $J = 7.3$ Hz, 3H, CH₃); ¹³C NMR (50 MHz, CDCl₃): 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 + NH₄]⁺, 100); Anal. Calcd for C₁₇H₂₆O₄: 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; ¹H NMR (200 MHz, CDCl₃): δ 7.64–7.22 (m, 9H, arom), 4.25 (q, $J = 4.0$ Hz, 1H, CH), 3.77 (s, 3H, OCH₃), 2.84 (t, $J = 8.2$ Hz, 2H, PhCH₂), 2.30–1.84

(m, 2H, CH₂); ¹³C NMR (50 MHz, CDCl₃): 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 + NH₄]⁺, 100); Anal. Calcd for C₁₇H₁₈O₃: 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 CH₂Cl₂ (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 CH₂Cl₂ (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, CDCl₃): 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, CH₂O), 3.77 (s, 3H, OCH₃), 2.58 (t, *J* = 7.3 Hz, 2H, PhCH₂), 1.94–1.40 (m, 10H, CH₂), 0.99 (t, *J* = 7.3 Hz, 3H, CH₃); ¹³C NMR (50 MHz, CDCl₃): 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; ¹⁹F NMR (188 MHz, CDCl₃): δ -150.03 (qt, *J* = 24.4 Hz). MS (ESI) *m/z* (%): 314 ([M + NH₄]⁺, 100); Anal. Calcd for C₁₇H₂₃FO₃: 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 H₂O (10 mL) and extracted with Et₂O (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 Na₂SO₄ and concentrating in vacuo.

6-(4-Butoxyphenyl)-2-hydroxyhexanoic Acid (13). Yield 95%; yellowish solid; mp 88–90 °C; ¹H NMR (200 MHz, CDCl₃): 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, CH₂O), 2.55 (t, *J* = 7.2 Hz, 2H, PhCH₂), 1.95–1.37 (m, 10H, 5 × CH₂), 0.97 (t, *J* = 6.4 Hz, 3H, CH₃); ¹³C NMR (50 MHz, CDCl₃): 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 C₁₆H₂₄O₄: 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, CDCl₃): 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, CH₂O), 2.58 (t, *J* = 7.2 Hz, 2H, PhCH₂), 2.06–1.36 (m, 10H, 5 × CH₂), 0.98 (t, *J* = 7.3 Hz, 3H, CH₃); ¹³C NMR (50 MHz, CDCl₃): 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; ¹⁹F NMR (188 MHz, CDCl₃): δ -150.4 (qt, *J* = 25.6 Hz). MS (ESI) *m/z* (%): 281 ([M - H]⁻, 100); Anal. Calcd for C₁₆H₂₃FO₃: 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, CDCl₃): δ 7.01 (d, *J* = 7.9 Hz, 2H, arom), 6.76 (d, *J* = 8.4 Hz, 2H, arom), 3.90 (m, 2H, CH₂O), 2.70–2.33 (m, 2H, PhCH₂), 2.15 (d, *J* = 6.6 Hz, 2H, CH₂CO), 1.85–1.10 (m, 10H, CH₂), 0.99 (t, *J* = 5.6 Hz, 3H, CH₃); ¹³C NMR (50 MHz, CDCl₃): 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 C₁₆H₂₄O₃: 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, CDCl₃): δ 7.64–7.19 (m, 9H, arom), 4.18–4.10 (m, 1H, CH), 2.80 (t, *J* = 7.4 Hz, 2H, PhCH₂), 2.23–1.83 (m, 2H, CH₂); ¹³C NMR (50 MHz, CDCl₃): 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 C₁₆H₁₆O₃: 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 CsCO₃ (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 (Na₂SO₄) 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, CDCl₃): 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, CH₂OCO), 3.92 (t, *J* = 6.4 Hz, 2H, CH₂O), 2.81 (d, *J* = 5.8 Hz, 1H, OH), 2.60–2.48 (m, 2H, PhCH₂), 2.24 (t, *J* = 6.9 Hz, 2H, CH₂CO), 1.86–1.36 [m, 23H, 7 × CH₂, C(CH₃)₃], 0.95 (t, *J* = 7.3 Hz, 3H, CH₃); ¹³C NMR (50 MHz, CDCl₃): 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 C₂₅H₄₀O₆: 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, CDCl₃): 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, CHOH, CH₂O), 3.91 (t, *J* = 6.5 Hz, 2H, OCH₂), 2.82 (brs, 1H, OH), 2.54 (t, *J* = 7.0 Hz, 2H, PhCH₂), 2.41 (t, *J* = 7.0 Hz, 2H, CH₂CO), 1.89–1.10 [m, 27H, 9 × CH₂, C(CH₃)₃], 0.89 (t, *J* = 6.6 Hz, 3H, CH₃); ¹³C NMR (50 MHz, CDCl₃): 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 C₂₇H₄₄O₆: C, 69.79; H, 9.55; found: C, 69.62; H, 9.72.

***tert*-Butyl 5-((4-([1,1'-Biphenyl]-4-yl)-2-hydroxybutanoyloxy)pentanoate (23c).** Yield 36%; colorless oil; ¹H NMR (200 MHz, CDCl₃): δ 7.62–7.22 (m, 9H, arom), 4.28–4.04 (m, 3H, OCH₂, CH), 2.82–2.78 (m, 3H, PhCH₂, OH), 2.33–1.94 (m, 4H, CH₂), 1.77–1.57 (m, 4H, 2 × CH₂), 1.44 (s, 9H, C(CH₃)₃); ¹³C NMR (50 MHz, CDCl₃): 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 + NH₄]⁺, 100); Anal. Calcd for C₂₃H₃₂O₅: 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

(200 MHz, CDCl₃): δ 7.64–7.22 (m, 9H, arom), 4.27–4.05 (m, 3H, OCH₂, CH), 2.83–2.78 (m, 3H, PhCH₂, OH), 2.30 (t, J = 7.2 Hz, 2H, CH₂CO), 2.09–1.86 (m, 4H, 2 \times CH₂), 1.44 [m, 9H, C(CH₃)₃]; ¹³C NMR (50 MHz, CDCl₃): 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 C₂₄H₃₀O₅: 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; ¹H NMR (200 MHz, CDCl₃): 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, CH₂OCO), 3.92 (t, J = 6.5 Hz, 1H, CH₂O), 2.60–2.45 (m, 1H, PhCH₂), 2.24 (t, J = 6.6 Hz, 2H, CH₂CO), 2.01–1.23 [m, 23H, 7 \times CH₂, C(CH₃)₃], 0.96 (t, J = 7.3 Hz, 3H, CH₃); ¹³C NMR (50 MHz, CDCl₃): 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; ¹⁹F NMR (188 MHz, CDCl₃): δ -149.9 (qt, J = 24.4 Hz); MS (ESI) m/z (%): 456 ([M + NH₄]⁺, 100); Anal. Calcd for C₂₅H₃₉FO₅: 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; ¹H NMR (200 MHz, CDCl₃): δ 7.03 (d, J = 8.5 Hz, 2H, arom), 6.78 (d, J = 8.6 Hz, 2H, arom), 4.05 (m, 2H, CH₂OCO), 3.89 (t, J = 6.4 Hz, 2H, CH₂O), 2.51 (t, J = 7.5 Hz, 2H, PhCH₂), 2.33–2.10 (m, 4H, 2 \times CH₂CO), 1.81–1.16 [m, 23H, 7 \times CH₂, C(CH₃)₃], 0.94 (t, J = 7.3 Hz, 3H, CH₃). ¹³C NMR (50 MHz, CDCl₃): 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 C₂₅H₄₀O₅: 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 CH₂Cl₂ (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 Na₂S₂O₃ 10% and NaHCO₃ 10% (15 mL, 1:1, v/v) and then with brine, dried over Na₂SO₄, 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; ¹H NMR (200 MHz, CDCl₃): 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, CH₂OCOCO), 3.91 (t, J = 6.4 Hz, 2H, CH₂O), 2.83 (t, J = 6.5 Hz, 2H, CH₂COCO), 2.55 (t, J = 6.6 Hz, 2H, PhCH₂), 2.25 (t, J = 6.8 Hz, 2H, CH₂COO), 1.82–1.23 [m, 21H, 6 \times CH₂, C(CH₃)₃], 0.95 (t, J = 7.3 Hz, 3H, CH₃); ¹³C 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 + NH₄]⁺, 100); Anal. Calcd for C₂₅H₃₈O₆: 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; ¹H NMR (200 MHz, CDCl₃): 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, CH₂OCO), 3.91 (t, J = 6.5 Hz, 2H, OCH₂), 2.83 (t, J = 6.0 Hz, 2H, CH₂COCO), 2.55 (t, J = 6.2 Hz, 2H, PhCH₂), 2.25 (t, J = 7.1 Hz, 2H, CH₂COO), 1.84–1.53 (m, 10H, CH₂), 1.43 [s, 9H,

C(CH₃)₃], 1.38–1.19 (m, 6H, CH₂), 0.89 (t, J = 6.3 Hz, 3H, CH₃); ¹³C NMR (50 MHz, CDCl₃): 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 + NH₄]⁺, 100); Anal. Calcd for C₂₇H₄₂O₆: C, 70.10; H, 9.15; found: C, 69.91; H, 9.32.

tert-Butyl 5-((4-([1,1'-Biphenyl]-4-yl)-2-oxobutanoyloxy)pentanoate (24c). Yield 70%; yellowish oil; ¹H NMR (200 MHz, CDCl₃): δ 7.67–7.22 (m, 9H, arom), 4.25 (t, J = 6.2 Hz, 2H, CH₂OCOCO), 3.22 (t, J = 6.9 Hz, 2H, CH₂COCO), 3.00 (t, J = 7.1 Hz, 2H, PhCH₂), 2.26 (t, J = 7.1 Hz, 2H, CH₂CO), 1.84–1.57 (m, 4H, 2 \times CH₂), 1.44 [s, 9H, C(CH₃)₃]; ¹³C NMR (50 MHz, CDCl₃): 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 + NH₄]⁺, 100); Anal. Calcd for C₂₅H₃₀O₅: 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; ¹H NMR (200 MHz, CDCl₃): δ 7.65–7.21 (m, 9H, arom), 4.28 (t, J = 6.4 Hz, 2H, CH₂OCOCO), 3.22 (t, J = 6.9 Hz, 2H, CH₂COCO), 2.99 (t, J = 7.2 Hz, 2H, PhCH₂), 2.33 (t, J = 7.2 Hz, 2H, CH₂CO), 2.09–1.91 (m, 2H, CH₂), 1.43 [s, 9H, 3 \times CH₃]; ¹³C NMR (50 MHz, CDCl₃): 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 + NH₄]⁺, 100); Anal. Calcd for C₂₄H₂₈O₅: C, 72.71; H, 7.12; found: C, 72.50; H, 7.34.

Methyl 6-(4-Butoxyphenyl)-2-oxohexanoate (28). Yield 60%; colorless oil; ¹H NMR (200 MHz, CDCl₃): 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, CH₂O), 3.85 (s, 3H, CH₃O), 2.86 (t, J = 6.9 Hz, 2H, CH₂CO), 2.57 (t, J = 6.9 Hz, 2H, PhCH₂), 1.82–1.40 (m, 8H, 4 \times CH₂), 0.97 (t, J = 7.3 Hz, 3H); ¹³C NMR (50 MHz, CDCl₃): 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 + NH₄]⁺, 100); Anal. Calcd for C₁₇H₂₄O₄: 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 CH₂Cl₂ (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 (CH₂Cl₂–MeOH, 95:5).

5-((6-(4-Butoxyphenyl)-2-oxohexanoyloxy)pentanoic Acid (25a). Yield 72%; white solid; mp 55–58 °C. ¹H NMR (200 MHz, CDCl₃): 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, CH₂OCO), 3.93 (t, J = 6.5 Hz, 2H, CH₂O), 2.84 (t, J = 6.8 Hz, 2H, CH₂COCO), 2.57 (t, J = 6.8 Hz, 2H, PhCH₂), 2.41 (t, J = 6.8 Hz, 2H, CH₂COOH), 1.83–1.38 (m, 12H, 6 \times CH₂), 0.96 (t, J = 7.3 Hz, 3H); ¹³C NMR (50 MHz, CDCl₃): 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 C₂₁H₂₉O₆[–] 377.1970; found 377.1961; Anal. Calcd for C₂₁H₃₀O₆: C, 66.65; H, 7.99; found: C, 66.37; H, 8.16.

5-((6-(4-(Hexyloxy)phenyl)-2-oxohexanoyloxy)pentanoic Acid (25b). Yield 84%; white solid; mp 69–71 °C; ¹H NMR (200 MHz, CDCl₃): δ 7.06 (d, J = 8.3 Hz, 2H, arom), 6.80 (d,

$J = 8.6$ Hz, 2H, arom), 4.25 (t, $J = 5.6$ Hz, 2H, CH₂OCO), 3.91 (t, $J = 6.5$ Hz, 2H, OCH₂), 2.84 (t, $J = 5.7$ Hz, 2H, CH₂COCO), 2.55 (t, $J = 6.7$ Hz, 2H, PhCH₂), 2.40 (t, $J = 6.6$ Hz, 2H, CH₂COOH), 1.94–1.09 (m, 16H, CH₂), 0.89 (t, $J = 5.6$ Hz, 3H, CH₃); ¹³C NMR (50 MHz, CDCl₃): 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 C₂₃H₃₃O₆[–] 405.2283; found: 405.2253; Anal. Calcd for C₂₃H₃₄O₆: 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; ¹H NMR (200 MHz, CDCl₃): δ 7.65–7.14 (m, 9H, arom), 4.26 (t, $J = 6.1$ Hz, 2H, CH₂OCOCO), 3.22 (t, $J = 6.5$ Hz, 2H, CH₂CO), 3.00 (t, $J = 7.2$ Hz, 2H, PhCH₂), 2.40 (t, $J = 6.9$ Hz, 2H, CH₂COOH), 1.90–1.59 (m, 4H, CH₂); ¹³C NMR (50 MHz, CDCl₃): 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 C₂₁H₂₁O₅[–] 353.1394; found: 353.1388; Anal. Calcd for C₂₁H₂₂O₅: 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; ¹H NMR (200 MHz, CDCl₃): δ 7.63–7.20 (m, 9H, arom), 4.30 (t, $J = 6.1$ Hz, 2H, CH₂OCOCO), 3.20 (t, $J = 7.4$ Hz, 2H, CH₂CO), 2.99 (t, $J = 7.2$ Hz, 2H, PhCH₂), 2.48 (t, $J = 7.2$ Hz, 2H, CH₂COOH), 2.14–1.96 (m, 2H, CH₂); ¹³C NMR (50 MHz, CDCl₃): 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 C₂₀H₁₉O₅[–] 339.1238; found: 339.1234; Anal. Calcd for C₂₀H₂₀O₅: 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; ¹H NMR (200 MHz, CDCl₃): 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, CH₂OCO), 3.93 (t, $J = 6.5$ Hz, 2H, CH₂O), 2.56 (t, $J = 7.3$ Hz, 2H, PhCH₂), 2.40 (t, $J = 6.6$ Hz, 2H, CH₂COOH), 2.07–1.35 (m, 14H, 7 × CH₂), 0.97 (t, $J = 7.3$ Hz, 3H, CH₃); ¹³C NMR (50 MHz, CDCl₃): 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; ¹⁹F NMR (188 MHz, CDCl₃): δ –149.9 (qt, $J = 24.4$ Hz); HRMS (ESI) m/z : [M – H][–] Calcd for C₂₁H₃₀FO₅[–] 381.2083; found: 381.2080; Anal. Calcd for C₂₁H₃₁FO₅: 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; ¹H NMR (200 MHz, CDCl₃): 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, CH₂OCO), 3.93 (t, $J = 6.5$ Hz, 2H, CH₂O), 2.56 (t, $J = 6.0$ Hz, 2H, PhCH₂), 2.45–2.22 (m, 4H, 2 × CH₂), 1.85–1.20 (m, 14H, 7 × CH₂), 0.97 (t, $J = 7.3$ Hz, 3H, CH₃); ¹³C NMR (50 MHz, CDCl₃): 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 C₂₁H₃₁O₅[–] 363.2177; found: 363.2174; Anal. Calcd for C₂₁H₃₂O₅: 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 Cs₂CO₃ (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 H₂O (10 mL) and extracted with Et₂O (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 Na₂SO₄ and concentrating in vacuo. Yield 94%; white solid; mp 102–105 °C; ¹H NMR (200 MHz, CDCl₃): 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, CH₂O), 2.94–2.61 (m, 1H, CHH), 2.57–2.21 (m, 3H, PhCH₂, CHH), 1.83–1.18 (m, 8H, 4 × CH₂), 0.97 (t, $J = 7.3$ Hz, 3H); ¹³C NMR (50 MHz, CDCl₃): 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 C₁₆H₂₁O₄[–] 277.1445; found: 277.1444; Anal. Calcd for C₁₆H₂₂O₄: C, 69.04; H, 7.97; found: C, 68.89; H, 8.21.

In Vitro PLA₂ Activity Assays. Group-specific mixed micelle modified Dole assays were employed to determine the activity of human recombinant GIVA cPLA₂, GVIA iPLA₂, and GV sPLA₂.^{18–20} To achieve optimum activity, the substrate was prepared using slightly different conditions for each enzyme: (i) GIVA cPLA₂ mixed micelle substrate consisted of 400 μM Triton X-100, 95.3 μM PAPC, 1.7 μM arachidonyl-1-¹⁴C PAPC, and 3 μM PIP₂ in a buffer containing 100 mM N-(2-hydroxyethyl)piperazine-*N'*-ethanesulfonic acid (HEPES) pH 7.5, 90 μM CaCl₂, 2 mM dithiothreitol (DTT), and 0.1 mg/mL bovine serum albumin; (ii) GVIA iPLA₂ mixed micelle substrate consisted of 400 μM Triton X-100, 98.3 μM PAPC, and 1.7 μM arachidonyl-1-¹⁴C PAPC in a buffer containing 100 mM HEPES pH 7.5, 2 mM adenosine 5'-triphosphate, and 4 mM DTT; and (iii) GV sPLA₂ mixed micelles substrate consisted of 400 μM Triton X-100, 98.3 μM PAPC, and 1.7 μM arachidonyl-1-¹⁴C PAPC in a buffer containing 50 mM Tris-HCl pH 8.0, and 5 mM CaCl₂. Initially, the compounds were screened at 0.091 mol fraction (5 μL of 5 mM inhibitor in dimethyl sulfoxide) in substrate (495 μL). X₁(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 X₁(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 mm² 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: H₂O/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.

■ 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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■ REFERENCES

- (1) Dennis, E. A.; Cao, J.; Hsu, Y. H.; Magrioti, V.; Kokotos, G. Phospholipase A₂ enzymes: Physical structure, biological function, disease implication, chemical inhibition, and therapeutic intervention. *Chem. Rev.* **2011**, *111*, 6130–6185.
- (2) Vasquez, A. M.; Mouchlis, D. V.; Dennis, E. A. Review of four major distinct types of human phospholipase A₂. *Adv. Biol. Regul.* **2018**, *67*, 212–218.
- (3) Dennis, E. A.; Norris, P. C. Eicosanoid storm in infection and inflammation. *Nat. Rev. Immunol.* **2015**, *15*, 511–523.
- (4) Leslie, C. C. Cytosolic phospholipase A₂: Physiological function and role in disease. *J. Lipid Res.* **2015**, *56*, 1386–1402.
- (5) Kokotou, M. G.; Limnios, D.; Nikolaou, A.; Psarra, A.; Kokotos, G. Inhibitors of phospholipase A₂ and their therapeutic potential: An update on patents (2012–2016). *Expert Opin. Ther. Pat.* **2017**, *27*, 217–225.
- (6) Ong, W. Y.; Farooqui, T.; Kokotos, G.; Farooqui, A. A. Synthetic and natural inhibitors of phospholipases A₂: Their importance for understanding and treatment of neurological disorders. *ACS Chem. Neurosci.* **2015**, *6*, 814–831.
- (7) Omland, S. H.; Habicht, A.; Damsbo, P.; Wilms, J.; Johansen, B.; Gniadecki, R. The role of IL-23 and the IL-23/T_H 17 immune axis in the pathogenesis and treatment of psoriasis. *J. Eur. Acad. Dermatol. Venereol.* **2017**, *31*, 1161–1167.
- (8) Bhowmick, R.; Clark, S.; Bonventre, J. V.; Leong, J. M.; McCormick, B. A. Cytosolic phospholipase A₂α promotes pulmonary inflammation and systemic disease during *Streptococcus pneumoniae* infection. *Infect. Immun.* **2017**, *85*, 280–317.
- (9) Tomoo, T.; Nakatsuka, T.; Katayama, T.; Hayashi, Y.; Fujieda, Y.; Terakawa, M.; Nagahira, K. Design, synthesis, and biological evaluation of 3-(1-aryl-1H-indol-5-yl)propanoic acids as new indole-

based cytosolic phospholipase A₂α inhibitors. *J. Med. Chem.* **2014**, *57*, 7244–7262.

(10) Kanai, S.; Ishihara, K.; Kawashita, E.; Tomoo, T.; Nagahira, K.; Hayashi, Y.; Akiba, S. ASB14780, an orally active inhibitor of group IVA phospholipase A₂, is a pharmacotherapeutic candidate for nonalcoholic fatty liver disease. *J. Pharmacol. Exp. Ther.* **2016**, *356*, 604–614.

(11) Kokotos, G.; Feuerherm, A. J.; Barbayianni, E.; Shah, I.; Sæther, M.; Magrioti, V.; Nguyen, T.; Constantinou-Kokotou, V.; Dennis, E. A.; Johansen, B. Inhibition of group IVA cytosolic phospholipase A₂ by thiazolyl ketones in vitro, ex vivo, and in vivo. *J. Med. Chem.* **2014**, *57*, 7523–7535.

(12) Kim, E.; Tunset, H. M.; Cebulla, J.; Vettukattil, R.; Helgesen, H.; Feuerherm, A. J.; Engebraten, O.; Mælandsmo, G. M.; Johansen, B.; Moestue, S. A. Anti-vascular effects of the cytosolic phospholipase A₂ inhibitor AVX235 in a patient-derived basal-like breast cancer model. *BMC Cancer* **2016**, *16*, 191.

(13) Li, Z.; Qu, M.; Sun, Y.; Wan, H.; Chai, F.; Liu, L.; Zhang, P. Blockage of cytosolic phospholipase A₂ alpha sensitizes aggressive breast cancer to doxorubicin through suppressing ERK and mTOR kinases. *Biochem. Biophys. Res. Commun.* **2018**, *496*, 153–158.

(14) Kokotou, M. G.; Galiatsatou, G.; Magrioti, V.; Koutoulogenis, G.; Barbayianni, E.; Limnios, D.; Mouchlis, V. D.; Satpathy, B.; Navratil, A.; Dennis, E. A.; Kokotos, G. 2-Oxoesters: A novel class of potent and selective inhibitors of cytosolic group IVA phospholipase A₂. *Sci. Rep.* **2017**, *7*, No. 7025.

(15) Crew, A. P.; Berlin, M.; Dong, H.; Qian, Y. Alanine-Based Modulators of Proteolysis and Associated Methods of Use. WO2017011590A1, 2017.

(16) Middleton, W. J. New fluorinating reagents. Dialkylaminosulfur fluorides. *J. Org. Chem.* **1975**, *40*, 574–578.

(17) Mceachern, E. J.; Sun, J.; Vocadlo, D. J.; Zhou, Y.; Zhu, Y.; Selnick, H. G. Glycosidase Inhibitors and Uses Thereof. WO201432188A1, 2014.

(18) Kokotos, G.; Six, D. A.; Loukas, V.; Smith, T.; Constantinou-Kokotou, V.; Hadjipavlou-Litina, D.; Kotsovolou, S.; Chiou, A.; Beltzner, C. C.; Dennis, E. A. Inhibition of group IVA cytosolic phospholipase A₂ by novel 2-oxoamides in vitro, in cells, and in vivo. *J. Med. Chem.* **2004**, *47*, 3615–3628.

(19) Stephens, D.; Barbayianni, E.; Constantinou-Kokotou, V.; Peristeraki, A.; Six, D. A.; Cooper, J.; Harkewicz, R.; Deems, R. A.; Dennis, E. A.; Kokotos, G. Differential inhibition of group IVA and group VIA phospholipases A₂ by 2-oxoamides. *J. Med. Chem.* **2006**, *49*, 2821–8.

(20) Six, D. A.; Barbayianni, E.; Loukas, V.; Constantinou-Kokotou, V.; Hadjipavlou-Litina, D.; Stephens, D.; Wong, A. C.; Magrioti, V.; Moutevelis-Minakakis, P.; Baker, S. F.; Dennis, E. A.; Kokotos, G. Structure-activity relationship of 2-oxoamide inhibition of group IVA cytosolic phospholipase A₂ and group V secreted phospholipase A₂. *J. Med. Chem.* **2007**, *50*, 4222–35.

(21) Lipinski, C.; Lombardo, F.; Dominy, B.; Feeney, P. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Delivery Rev.* **1997**, *23*, 3–25.

(22) Kokotos, G.; Hsu, Y.-H.; Burke, J. E.; Baskakis, C.; Kokotos, C. G.; Magrioti, V.; Dennis, E. A. Potent and selective fluoroketone inhibitors of group VIA calcium-independent phospholipase A₂. *J. Med. Chem.* **2010**, *53*, 3602–3610.

(23) Di, L.; Kerns, E. H.; Hong, Y.; Chen, H. Development and application of high throughput plasma stability assay for drug discovery. *Int. J. Pharm.* **2005**, *297*, 110–119.