3-(3,5-Dimethyl-4-octadecanoylpyrrol-2-yl)propionic Acids as Inhibitors of 85 kDa Cytosolic Phospholipase A_2

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Key Words: *3-(pyrrol-2-yl)propionic acids; 1-acylation of pyrroles; cytosolic phospholipase A_2 inhibitors*

Summary

3-(1,4-Diacylpyrrol-2-yl)propionic acids were designed as inhibitors of cytosolic phospholipase A_2. Enzyme inhibition was assayed by evaluation of calcium ionophore A23187-induced arachidonic acid release from bovine platelets. While the synthesized bisacyl compound 3-[3,5-dimethyl-4-octadecanoyl-1-(3-phenylpropionyl)pyrrol-2-yl]propionic acid was inactive at 33 μM, the related monoacylated 3-(3,5-dimethyl-4-octadecanoylpyrrol-2-yl)-propionic acid and 3-(1,3,5-trimethyl-4-octadecanoylpyrrol-2-yl)-propionic acid proved to be inhibitors of cytosolic phospholipase A_2 (IC_{50}: 24 μM and 13 μM, respectively).

Introduction

Phospholipases A_2 (PLA_2s) are a class of enzymes which catalyze the hydrolysis of membrane glycerophospholipids at the *sn*-2 position to release fatty acids and lysophospholipids. When the liberated fatty acid is arachidonic acid, subsequent metabolism by the cyclooxygenase and the 5-lipoxygenase leads to the formation of prostaglandins, which play a major part in the inflammatory response, and leukotrienes, which play a main role in the pathogenesis of asthma[1,2]. The other products of PLA_2 action are cytolytic lysophospholipids. From these the 1-*O*-alkyl-substituted lysophosphocholines can be further metabolized to the platelet activating factor (PAF). The lysophospholipids and the PAF are also potent mediators of inflammation[3,4].

Recently a 85 kDa cytosolic PLA_2 ($cPLA_2$) was isolated and purified[5–8] that is distinct from the well-characterized 14 kDa secretory PLA_2s from pancreas (type I $sPLA_2$) and blood cells (type II $sPLA_2$)[9]. This $cPLA_2$ seems to be the key control point for the biosynthesis of the lipid mediators mentioned above[10,11]. It selectively cleaves phospholipids containing arachidonic acid in *sn*-2 position contrary to the $sPLA_2$s, which do not show any degree of selectivity for the hydrolysis of arachidonic acid at the scissile ester position of the substrate. Moreover, $cPLA_2$ levels and activity are elevated upon treatment of cells with pro-inflammatory cytokinines[11–13]. Therefore inhibitors of $cPLA_2$ might become useful therapeutics for inflammatory diseases and asthma.

The therapeutically used nonsteroidal anti-inflammatory drugs (NSAID) such as aspirin and indomethacin are inhibitors of prostaglandin biosynthesis. However, the formation of pro-inflammatory lysophospholipids and PAF is not reduced by theses compounds. Thus since inhibitors of $cPLA_2$ block the synthesis of all mentioned lipid mediators, they are expected to have a better quality of action than the NSAIDs used today. Possibly the anti-inflammatory and anti-asthmatic potency of potent $cPLA_2$ inhibitors is similar to that of glucocorticoids, since the latter compounds exert their therapeutic effect at least in part by preventing activation of $cPLA_2$ by cytokinines[14–17].

Despite several inhibitors of $cPLA_2$ having been discovered, *e.g.* (*S*)-*N*-hexadecylpyrrolidine-2-carboxamide (Wy-48,489)[18–20] (**1**) and arachidonyl trifluoromethyl ketone ($AACOCF_3$)[21] (**2**) (Figure 1), no compound has been reported as undergoing clinical development.

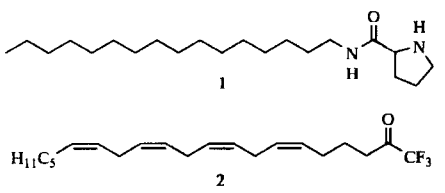

Figure 1

In our search for anti-inflammatory $cPLA_2$ inhibitors we designed compounds which show structural similarity to the enzyme substrates (**I**) and which are therefore probably able to inhibit substrate cleavage by binding to the active site of the enzyme. Such compounds were *e.g.* 3-(1,4-diacylpyrrol-2-yl)propionic acids (**II**) (Figure 2).

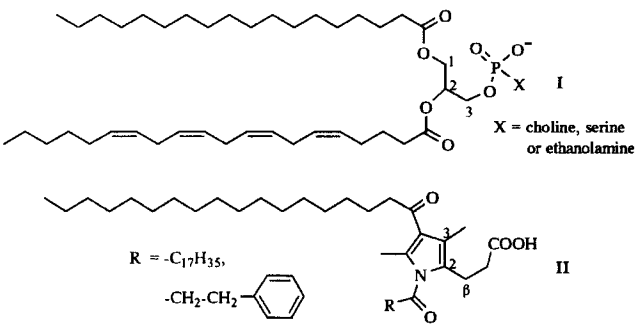

Figure 2

The C3 of the pyrrole, the C2 of the pyrrole and the β-C of the propionic acid side chain mimic the C1, C2, and C3 of the glycerol part of the phospholipids; the acyl residues in position 1 and 4 of the pyrrole imitate the two acyl residues of such phospholipids which contain an ester group in position 1 of the glycerol backbone (**I**); the phosphate group of the phospholipid is replaced by a bioisosteric carboxylate moiety[22,23]. For the acyl residue in position 1 of the pyrrole we chose an octadecanoyl and a 3-phenylpropionyl substituent,

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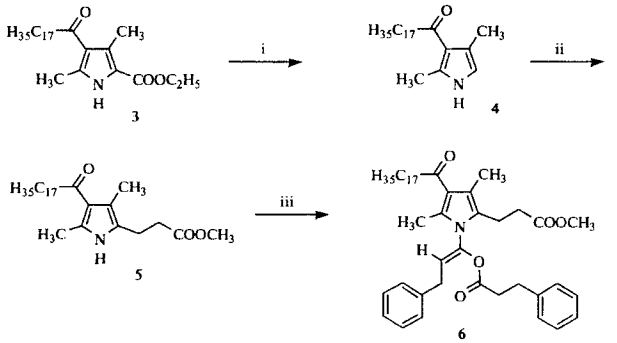
respectively. The latter one should imitate C1-C7 of the arachidonic acid of the natural substrate, whereby the C5-*cis* double bond is part of a phenyl ring. Regarding the structures of known $cPLA_2$ inhibitors[18–21] it was obvious that a polar head group like choline, ethanolamine, or serine is not essential for inhibitory activity. Initially, therefore, we did not introduce such a structural constituent of the phospholipids into the molecules. The potential pharmacophoric groups were affixed to a cycle, since incorporation of elements of the flexible substrate molecules into a rigid ring system may result in agents that show a higher degree of affinity to the enzyme than the natural substrates[24]. The pyrrole was selected as cycle because molecular modifications of this reactive ring system can be readily performed. To avoid the formation of isomers the synthesis started from pyrroles which contain methyl groups in positions 3 and 5.

While attempts to synthesize **II** with an octadecanoyl residue in position 1 of the pyrrole ring failed, we succeeded in preparing the derivative with an 1-(3-phenylpropionyl) moiety. Unfortunately, this compound (**II**, R = phenylethyl) was inactive at a concentration of 33 μM. However, the related monoacylated 3-(3,5-dimethyl-4-octadecanoylpyrrol-2-yl)propionic acid and 3-(1,3,5-trimethyl-4-octadecanoylpyrrol-2-yl)propionic acid inhibited $cPLA_2$ at concentrations less than 33 μM.

Synthesis

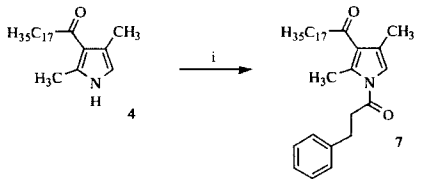
The synthesis of **II** started from ethyl 3,5-dimethyl-4-octadecanoylpyrrole-2-carboxylate (**3**) [25]. Saponification and thermal decarboxylation led to the octadecanoylpyrrole **4** (Scheme 1).



Scheme 1. (i) 1. KOH, H_2O, EtOH; 2. 160–170 °C; (ii) methyl acrylate, BF_3-Et_2O, nitrobenzene; (iii) 3-phenylpropionyl chloride, $(CH_4)_4N^+Br^-$, powdered NaOH, Et_2O.

The introduction of the methyl propionate chain succeeded by reaction with methyl acrylate, BF_3 in nitrobenzene. In the next step the received pyrrole ester **5** should be acylated in position 1 by phase transfer reaction with acyl chloride, tetrabutylammonium bromide and powdered sodium hydroxide in Et_2O. When reacting **5** with 3-phenylpropionyl chloride in the same molar ratio TLC control of the reaction course indicated that smaller amounts of a product were formed, but upon work-up almost solely the starting pyrrole was isolated. Using a threefold excess of the 3-phenylpropionyl chloride a product could be separated. However, not the desired monobut a bisacylated compound (**6**) was afforded, formed by further *O*-acylation of the enol tautomer of the initially formed monoacyl product (Scheme 1). Similar bisacylations of pyrroles have already been described in literature[26]. The isolated compound **6** possesses the *Z*-configuration, as established by ^1H NMR nuclear Overhauser enhancement (NOE) experiments: the NOE difference ^1H NMR spectrum upon irradiation of the methyl group in position 5 of the pyrrole exhibited a NOE at the hydrogen in position 2 of the 1-(prop-1-en-1-yl) substituent. So this proton could get closer to the 5-methyl group of the pyrrole than the two protons in the neighbouring position. These findings indicated that **6** had the *Z*-configuration.

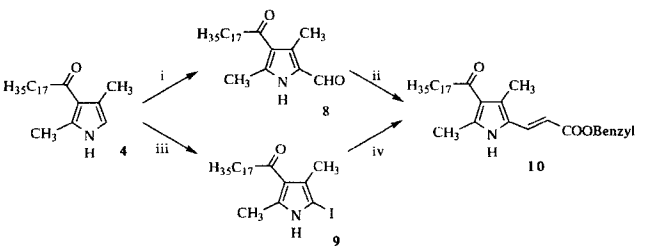
Surprisingly the acylpyrrole without a propionate side chain (**4**) could be monoacylated in good yields by phase transfer reaction to form **7** (Scheme 2).



Scheme 2. (i) 3-phenylpropionyl chloride, $(CH_4)_4N^+Br^-$, powdered NaOH, Et_2O.

Thus it was possible that interaction of the proton in position 5 of the pyrrole **4** with the carbonyl moiety of the acyl residue stabilizes the monoacyl adduct **7** or that the propionate side chain of **5** favours the formation of the bisacyl product by the additional +*I* effect of its β-C.

For studying the influence of the *I* effect of the ester side chain on the acylation behaviour we replaced the methyl propionate by a benzyl acrylate group (**10**), which exerts an –*I* effect on the pyrrole ring system. In order to synthesize **10** the pyrrole **4** was formylated by the Vilsmeier-Haack method to yield the carbaldehyde **8** which then was converted to **10** by Wittig reaction (Scheme 3). Alternatively **10** could be received by Heck reaction from the iodopyrrole **9** following known procedures [27,28]. However, the overall yield was lower using this reaction sequence.

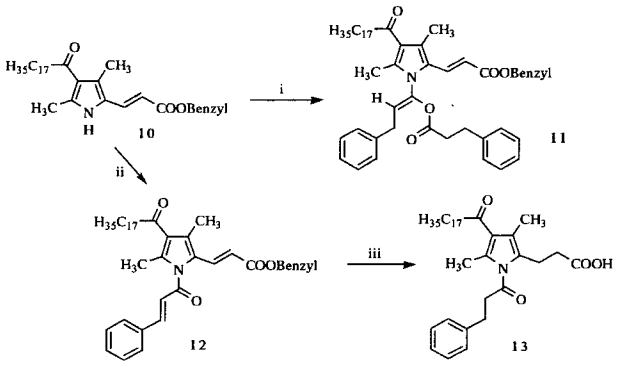


Scheme 3. (i) DMF, $POCl_3$, benzene; (ii) benzyloxycarbonylmethyltriphenylphosphonium bromide, benzene, CH_2Cl_2; (iii) aqueous I_2/KI, K_2CO_3, Et_2O, MeOH; (iv) benzyl acrylate, Pd(II)acetate, acetonitrile, triethylamine.

Instead of the pyrrole methyl acrylate corresponding to the methyl propionate **5** we synthesized the benzyl acrylate **10**, since in case of a successful monoacylation with 3-phenylpropionyl chloride this derivative could have been converted in only one step to the desired propionic acid derivative **II** by

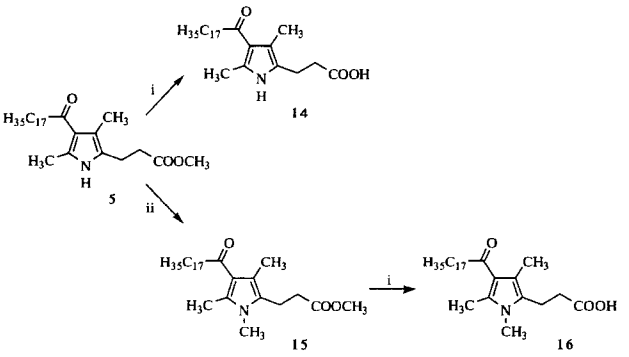
catalytic hydrogenation. However, reaction with an excess of 3-phenylpropionyl chloride yielded also a bisacylation product (**11**) (Scheme 4), so stabilisation of the carbonyl group of the 1-acyl moiety by an interaction with the α-hydrogen atom of the pyrrole nucleus seems to be the reason that **4** was only monoacylated.

Finally, the benzyl acrylate **10** was reacted with cinnamoyl chloride, which is not enolizable because of its α-double bond. In this case only monoacylation took place (Scheme 4). The received compound **12** could be converted to the desired 3-(1,4-diacylpyrrol-2-yl)propionic acid **13** by catalytic hydrogenation. Attempts to synthesize the 1-octadecanoyl derivative in the same way by using octadec-2-enoyl chloride failed.



Scheme 4. (i) 3-phenylpropionyl chloride, $(CH_4)_4N^+Br^-$, powdered NaOH, Et_2O; (ii) cinnamoyl chloride, $(CH_4)_4N^+Br^-$, powdered NaOH, Et_2O, CH_2Cl_2; (iii) H_2, Pd/C, ethyl acetate.

In order to synthesize 3-(3,5-dimethyl-4-octadecanoylpyrrol-2-yl)propionic acid (**14**) and 3-(1,3,5-trimethyl-4-octadecanoylpyrrol-2-yl)propionic acid (**16**) the intermediate **5** was saponified or *N*-methylated and saponified (Scheme 5).



Scheme 5. (i) KOH, H_2O, EtOH; (ii) methyl *p*-toluenesulfonate, $(CH_4)_4N^+Br^-$, powdered NaOH, Et_2O.

Results and Discussion

The biological activity of the test compounds was evaluated by measuring the calcium ionophore A23187-induced arachidonic acid release from bovine platelets with HPLC/UV-detection[29]. This assay detects inhibitors of the $cPLA_2$[30,31].

The 3-(1,4-diacylpyrrol-2-yl)propionic acid **13** did not inhibit $cPLA_2$ at a concentration of 33 μM.

The intermediate **5** showed some structural similarities to the known PLA_2 inhibitor 1-methyl-4-(2-naphthoyl)pyrrole-2-carboxylic acid (**17**) (Figure 3), which had been reported to inhibit calcium ionophore A23187-challenged arachidonic acid release in human monocytes with an IC_{50} of 210 μM[32].

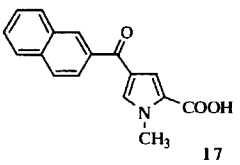
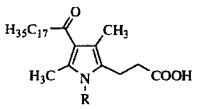


Figure 3

For this reason we also synthesized the carboxylic acid derivative and the *N*-methylated carboxylic acid derivative of **5**. Actually the received compounds **14** and **16** were inhibitors of $cPLA_2$ with IC_{50} of 24 μM and 13 μM, respectively (Table 1). The known $cPLA_2$ inhibitors Wy-48,489 (**1**) and AACOCF$_3$ (**2**) inhibited $cPLA_2$ in the same assay with an IC_{50} of 13 μM and 11 μM, respectively. The pyrrole carboxylic acid **17** proved to be less active. Its IC_{50} evaluated with bovine platelets was greater than 100 μM (24 % inhibition at 100 μM).

Table 1. *In vitro* data for 3-(pyrrol-2-yl)propionic acids and reference compounds.



Compound	R	Cell lysis at 33 μM [%]*	IC_{50} [μM]**
13	3-phenyl-propionyl	0	not active at 33 μM
14	H	0	24
16	CH_3	0	13
Wy-48,489 (**1**)		0	13
AACOCF$_3$ (**2**)		31	11
1-Methyl-4-(2-naphthoyl)pyrrole-2-carboxylic acid (**17**)		0 at 100 μM	>100

* Expressed as the decrease of absorbance of the cell suspension at 800 nm.
** Inhibitory potency on $cPLA_2$. Each value is the average of two runs and experimental error is within ±20%.

An explanation for the inactivity of the designed diacylpyrrole **13** may be that the rigidisation of the two acyl chains by the pyrrole ring system does not permit the molecule to adopt that conformation which is necessary to be bound to the

enzyme. Another possible reason for the negative test result can be found when regarding the structures of known $cPLA_2$ inhibitors[18–21,33]. Like the active 3-(pyrrol-2-yl)propionic acids **14** and **16** these compounds contain only one long acyl or alkyl residue. Thus the introduction of a second longer acyl or alkyl group into a $cPLA_2$ inhibitor molecule could principally cause the loss of activity.

Recently we have shown that $cPLA_2$ inhibition is faked when a substance leads to lysis of the platelets[33]. Therefore we measured the cell lytic potency of the test compounds by turbidimetry. With exception of the reference substance **2** none of the tested compounds caused cell lysis at the highest measured concentration of 33 µM (in case of **17**: 100 µM).

To exclude the possibility that our most active compound **16** acts by modifying the calcium ionophore A23187-induced activation mechanism of $cPLA_2$ and not by affecting the $cPLA_2$ directly we also stimulated the bovine platelets with 12-*O*-tetradecanoylphorbol-13-acetate (TPA). While calcium ionophore A23187 triggers activation of $cPLA_2$ by causing a calcium influx into the cells[34], TPA exerts its stimulatory effect in another way by causing phosphorylation of the enzyme as consequence of an activation of a protein kinase C (PKC) or a mitogen-activated protein kinase (MAP kinase)[35–37]. Since compound **16** proved to be active also in this assay, it can be assumed that it is actually an inhibitor of $cPLA_2$. The IC_{50} measured with TPA (24 µM) was higher than the IC_{50} received after calcium ionophore A23187 stimulation of the platelets (13 µM). The same effect was observed for the reference compound **1**[19,31].

No confident assertion can be made about the way of action of the compounds. Different mechanisms are possible: *e.g.* a compound can incorporate into the aggregated substrate assembly and may alter the physical properties of the membrane in such a way as to cause the enzyme to desorb from the surface and hence cause a change in enzyme rate[10], or it can act as allosteric inhibitor or it can interact directly with the active site of the enzyme.

Structure-activity relationship investigations with the developed lead structures **14** and **16** will be carried out in order to define their pharmacophoric groups and to gain a mechanistic insight into the basis of their $cPLA_2$ inhibitory activity in this way.

Acknowledgement

I thank Prof. Dr. H.-D. Stachel for supporting this study and Mrs. Monika Klimt for technical assistance.

Experimental

Chemistry

General

Melting points (uncorrected): Büchi melting point apparatus.– ^{1}H NMR spectra: Jeol JNM-GX 400 spectrometer (400 MHz); δ values in ppm relative to internal tetramethylsilane.– Mass spectra: Varian CH7 instrument; electron beam ionisation at 70 eV (EI) or chemical ionisation with methane (CI).– Elemental analyses: Heraeus CHN Rapid instrument.– Reference compounds: arachidonyl trifluoromethyl ketone was purchased from Biomol (Hamburg); (*S*)-*N*-hexadecylpyrrolidine-2-carboxamide and 1-methyl-4-(2-naphthoyl)pyrrole-2-carboxylic acid were synthesized by known procedures.

2,4-Dimethyl-3-octadecanoylpyrrole **4**

The mixture of ethyl 3,5-dimethyl-4-octadecanoylpyrrole-2-carboxylate[25] (**3**) (7.4 g, 17 mmol), EtOH (80 ml) and 20% aqueous KOH (30 ml) was refluxed for 2 h. The cooled reaction mixture was diluted with water, acidified with dilute HCl and extracted twice with $CHCl_3$. The organic layers were evaporated and the residue heated in an oil bath at 160–170 °C for 20 min. Purification by chromatography on silica gel with CH_2Cl_2 and precipitation from MeOH yielded **4** (3.3 g, 54 %) as solid. Mp 70–72 °C.– ^{1}H NMR ($CDCl_3$): δ (ppm) = 0.88 (t, *J* = 7 Hz, 3H, CH_3), 1.15-1.41 [m, 28H, $(CH_2)_{14}$], 1.68 (quint, *J* = 7 Hz, 2H, CH_2CH_2CO), 2.28 (s, 3H, $PyrCH_3$), 2.50 (s, 3H, $PyrCH_3$), 2.70 (t, *J* = 7 Hz, 2H, CH_2CO), 6.36 (s, 1H, aromatic H), 7.92 (s, 1H, NH).– MS (EI): *m/z* (%) = 361 (8) [M^+], 122 (100).

Methyl 3-(3,5-dimethyl-4-octadecanoylpyrrol-2-yl)propionate **5**

To the solution of **4** (1.0 g, 2.8 mmol) and methyl acrylate (1.0 ml) in dry nitrobenzene (10 ml) boron trifluoride diethyl ether complex (0.4 ml) was added. After being stirred for 36 h the mixture was poured into brine and extracted with Et_2O. The organic phase was dried (Na_2SO_4) and evaporated. The residue was chromatographed on silica gel with petroleum ether-ethyl acetate (1. 9+1, 2. 7+3). Evaporation of the eluate gave **5** (0.90 g, 72 %) as solid. Mp 82–83 °C.– ^{1}H NMR ($CDCl_3$): δ (ppm) = 0.88 (t, *J* = 7 Hz, 3H, CH_3), 1.14–1.44 [m, 28H, $(CH_2)_{14}$], 1.67 (quint, *J* = 7 Hz, 2H, CH_2CH_2COPyr), 2.18 (s, 3H, $PyrCH_3$), 2.46 (s, 3H, $PyrCH_3$), 2.56 (t, *J* = 6 Hz, 2H, CH_2), 2.68 (t, *J* = 7 Hz, 2H, CH_2COPyr,), 2.79 (t, *J* = 6 Hz, 2H, CH_2), 3.70 (s, 3H, OCH_3), 8.51 (s, 1H, NH).– MS (EI): *m/z* (%) = 447 (21) [M^+], 208 (100), 176 (54).

(Z)-Methyl 3-[3,5-dimethyl-4-octadecanoyl-1-[3-phenyl-1-(3-phenylpropionyloxy)prop-1-en-1-yl]pyrrol-2-yl]propionate **6**

The solution of 3-phenylpropionyl chloride (34 mg, 0.2 mmol) in Et_2O (2 ml) was added to a mixture of **5** (90 mg, 0.2 mmol), tetrabutylammonium bromide (32 mg, 0.1 mmol), powdered NaOH (30 mg, 0.75 mmol) and dry Et_2O (3 ml). The resulting mixture was refluxed for 30 min, whereby a solution of 3-phenylpropionyl chloride (34 mg, 0.2 mmol) in Et_2O (2 ml) was added after 10 min and 20 min each time. The mixture was filtered and the organic phase evaporated. Purification of the residue by chromatography on silica gel with petroleum ether-ethyl acetate 9+1 yielded **6** (93 mg, 65 %) as oil.^{1}H NMR: δ (ppm) = 0.88 (t, *J* = 7 Hz, 3H, CH_3), 1.18–1.42 [m, 28H, $(CH_2)_{14}$], 1.66 (quint, *J* = 7 Hz, 2H, CH_2CH_2CO), 2.16 (s, 3H, $PyrCH_3$), 2.38–2.45 (m, 2H, CH_2), 2.47 (s, 3H, $PyrCH_3$), 2.68 (t, *J* = 7 Hz, 2H, CH_2), 2.75 (t, *J* = 8 Hz, 2H, CH_2), 2.90–2.97 (m, 4H, CH_2 and CH_2), 3.31 (d, *J* = 7 Hz, $C{=}CHCH_2Phenyl$), 3.67 (s, 3H, OCH_3), 5.46 (t, *J* = 7 Hz, 1H, $C{=}CH{-}CH_2Phenyl$), 7.09-7.32 (m, 5H, aromat.H).– ^{1}H NMR-NOE difference spectrum: positive NOE-effect at 5.46 ppm when irradiating at 2.47 ppm.– MS (EI): *m/z* (%) = 712 (4) [M^+], 711 (9), 447 (100), 223 (69), 208 (62).

2,4-Dimethyl-3-octadecanoyl-1-(3-phenylpropionyl)pyrrole **7**

The preparation of **7** followed the procedure described for **6** using **4** (72 mg, 0.2 mmol) instead of **5**. Purification by chromatography yielded **7** (89 mg, 72 %) as solid. Mp 70–71 °C.– ^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.88 (t, *J* = 7 Hz, 3H, CH_3), 1.18–1.42 [m, 28H, $(CH_2)_{14}$], 1.66 (quint, *J* = 7 Hz, 2H, CH_2CH_2CO), 2.15 (s, 3H, $PyrCH_3$), 2.69 (s, 3H, $PyrCH_3$), 2.70 (t, *J* = 7 Hz, 2H, CH_2CH_2CO), 3.04–3.15 (m, 4H, $COCH_2CH_2Phenyl$), 6.77 (s, 1H, PyrH), 7.14–7.33 (m, 5H, aromat.H).– MS (EI): *m/z* (%) = 493 (4) [M^+], 263 (21), 248 (21), 137 (94), 122 (100).

3,5-Dimethyl-4-octadecanoylpyrrole-2-carbaldehyde **8**

Dry DMF (1.32 g, 18 mmol) was cooled in an ice bath and treated with $POCl_3$ (0.91 g, 6 mmol). The mixture was stirred at 0 °C for 30 min. Then the solution of **4** (2.17 g, 6 mmol) in dry benzene (20 ml) was added and the mixture was stirred at room temp. for 2 h. After adding the solution of sodium acetate (2.5 g) in water (10 ml) the reaction mixture was refluxed with vigorous stirring. The cooled reaction mixture was diluted with water and extracted twice with Et_2O/CH_2Cl_2 (3+1). The organic layers were dried (Na_2SO_4) and evaporated. Purification of the residue by chromatography on silica gel with CH_2Cl_2-ethyl acetate 9+1 and precipitation from petroleum ether yielded **8** (1.4 g, 60 %) as solid. Mp 96–97 °C.– ^{1}H-NMR ($CDCl_3$): δ

(ppm) = 0.88 (t, J = 7 Hz, 3H, CH_3,), 1.12–1.42 [m, 28H, $(CH_2)_{14}$], 1.62 (quint, J = 7 Hz, 2H, CH_2CH_2CO), 2.57 (s, 6H, PyrCH$_3$ and PyrCH$_3$), 2.72 (t, J = 7 Hz, 2H, CH_2CO), 9.65 (s, 1H, CHO).– MS (EI): *m/z* (%) = 389 (3) [M^+], 150 (100).

2-Iodo-3,5-dimethyl-4-octadecanoylpyrrole **9**

To the mixture of **4** (2.17 g, 6 mmol), K_2CO_3 (2.1 g, 15 mmol), Et_2O (60 ml) and MeOH (30 ml) was added 0.05 M I_2/KI-solution (180 ml) and the resulting mixture was stirred at room temp. for 15 min. The reaction mixture was diluted with Et_2O/CH_2Cl_2 (2+1, 300 ml) and washed with the solution of $Na_2S_2O_3$ (1.6 g, 10 mmol) in water (300 ml). The organic layer was dried (Na_2SO_4) and evaporated. The residue was chromatographed on silica gel with petroleum ether-ethyl acetate 9+1. Evaporation of the eluate yielded **9** (1.33 g, 45 %) as solid. Mp 76–78 °C.– ^{1}H NMR ($CDCl_3$): δ (ppm) = 0.88 (t, J = 7 Hz, 3H, CH_3), 1.18-1.40 [m, 28H, $(CH_2)_{14}$], 1.67 (quint, J = 7 Hz, 2H, CH_2CH_2CO), 2.24 (s, 3H, PyrCH$_3$), 2.50 (s, 3H, PyrCH$_3$), 2.69 (t, J = 7 Hz, 2H, CH_2CH_2CO), 7.90 (broad, 1H, NH).– MS (EI): *m/z* (%) = 487 (11) [M^+], 248 (100), 137 (62), 122 (93).

(E)-Benzyl 3-(3,5-dimethyl-4-octadecanoylpyrrol-2-yl)acrylate **10**
Method A:

The mixture of triphenylphosphine (2.36 g, 9 mmol), benzyl bromoacetate (2.07 g, 9 mmol) and dry benzene (15 ml) was stirred for 10 min at room temp. and then refluxed for 30 min. After cooling to ambient temp. water (15 ml), toluene (15 ml) and CH_2Cl_2 (30 ml) were added and the mixture was made alkaline under vigorous stirring with 2N NaOH using phenolphthalein as indicator. The organic phase was dried (Na_2SO_4) and evaporated. To the residue the solution of **8** (1.36 g, 3.5 mmol) in dry benzene (40 ml) and dry CH_2Cl_2 (20 ml) was added and the mixture was stirred for 24 h. The reaction mixture was diluted with CH_2Cl_2 and washed with water. The organic layer was dried (Na_2SO_4) and evaporated. The residue was chromatographed on silica gel with petroleum ether-ethyl acetate 9+1. Precipitation from petroleum ether yielded **10** (0.71 g, 39 %) as solid.

Method B:

The mixture of **9** (1.22 g, 2.5 mmol), acetonitrile (10 ml), triethylamine (0.80 ml), benzyl acrylate (0.80 ml) and palladium (II) acetate (20 mg) was heated in an oil bath at 90-100°C for 45 min. The cooled reaction mixture was diluted with water and extracted with CH_2Cl_2. The organic layer was dried (Na_2SO_4) and evaporated. Precipitation from petroleum ether yielded **10** (208 mg, 16%) as solid. Mp 121–123 °C.– ^{1}H NMR ($CDCl_3$): δ (ppm) = 0.88 (t, J = 7 Hz, 3H, CH_3), 1.17–1.39 [m, 28H, $(CH_2)_{14}$], 1.67 (quint, J = 7 Hz, 2H, CH_2CH_2CO), 2.36 (s, 3H, PyrCH$_3$), 2.53 (s, 3H, PyrCH$_3$), 2.70 (t, J = 7 Hz, 2H, CH_2CH_2CO), 5.24 (s, 2H, OCH_2Phenyl), 5.93 (d, J = 16 Hz, 1H, CH=CHCO), 7.28–7.41 (m, 5H, aromatic H), 7.65 (d, J = 16 Hz, 1H, CH=CHCO), 8.59 (broad, 1H, NH).– MS (EI): *m/z* (%) = 521 (21) [M^+], 297 (46), 282 (45), 174 (100).

(E)-Benzyl 3-{(Z)-3,5-dimethyl-4-octadecanoyl-1-[3-phenyl-1-(3-phenylpropionyloxy)prop-1-en-1-yl]pyrrol-2-yl}acrylate **11**

The preparation of **11** followed the procedure described for **6** using **10** (104 mg, 0.2 mmol) instead of **5**. To dissolve **10** CH_2Cl_2 (1.5 ml) was added to the reaction mixture besides. **11** was yielded as oil (78 mg, 50 %). ^{1}H NMR ($CDCl_3$): δ (ppm) = 0.88 (t, J = 7 Hz, 3H, CH_3), 1.16-1.37 [m, 28H, $(CH_2)_{14}$], 1.67 (quint, J = 7 Hz, 2H, CH_2CH_2CO), 2.35 (s, 3H, PyrCH$_3$), 2.48 (s, 3H, PyrCH$_3$), 2.70-2.75 (m, 4H, CH_2 and CH_2), 2.92 (t, J = 8 Hz, 2H, CH_2), 3.36 (d, J = 8 Hz, C=CHCH_2Phenyl), 5.25 (s, 2H, OCH_2Phenyl), 5.49 (t, J = 7 Hz, 1H, C=CHCH$_2$Phenyl), 6.14 (d, J = 16 Hz, 1H, CH=CHCO), 7.12-7.43 (m, 15H, aromat.H), 7.79 (d, J = 16 Hz, 1H, CH=CHCO).– ^{1}H NMR-NOE difference spectrum: positive NOE-effect at 5.49 ppm when irradiating at 2.48 ppm.– MS (CI): *m/z* (%) = 787 (2)[(M+1)$^+$], 655 (11), 523 (8), 83 (100).

(E)-Benzyl 3-{3,5-dimethyl-4-octadecanoyl-1-[(E)-3-phenylacryloyl]pyrrol-2-yl}acrylate **12**

Preparation analogous to that of **6** using **10** (104 mg, 0.2 mmol) instead of **5** and cinnamoyl chloride instead of 3-phenylpropionyl chloride yielded **12** (20 mg, 15 %) as solid. The yield could be raised from 15 % to 47 % when applying the following procedure:

The solution of cinnamoyl chloride (83 mg, 0.5 mmol) in Et_2O (2 ml) was added to a mixture of **10** (157 mg, 0.3 mmol), tetrabutylammonium bromide (48 mg, 0.15 mmol), powdered NaOH (300 mg, 7.5 mmol), CH_2Cl_2 (30 ml), Et_2O (10 ml) and water (3 drops). The resulting mixture was refluxed for 30 min, whereby a solution of cinnamoyl chloride (83 mg, 0.5 mmol) in Et_2O (2 ml) was added after 15 min. The mixture was diluted with water and extracted with Et_2O. The organic layer was washed with aqueous $NaHCO_3$ solution, dried (Na_2SO_4) and evaporated. Purification of the residue by chromatography on silica gel with petroleum ether-ethyl acetate 9+1 and precipitation from petroleum ether yielded **12** (91 mg, 47 %) as solid. Mp 76-78 °C.– ^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.88 (t, J = 7 Hz, 3H, CH_3), 1.15-1.39 [m, 28H, $(CH_2)_{14}$], 1.69 (quint, J = 7 Hz, 2H, CH_2CH_2CO), 2.36 (s, 3H, PyrCH$_3$), 2.51 (s, 3H, PyrCH$_3$), 2.75 (t, J = 7 Hz, 2H, CH_2CH_2CO), 5.14 (s, 2H, OCH_2Phenyl), 5.93 (d, J = 16 Hz, 1H, CH=CHCO), 6.76 (d, J = 16 Hz, 1H, CH=CHCO), 7.28-7.54 (m, 10H, aromat.H), 7.66 (d, J = 16 Hz, 2H, CH=CHCO and CH=CHCO).– MS (CI): *m/z* (%) = 652 (52)[(M+1)$^+$], 391 (43), 83 (100).

3-[3,5-Dimethyl-4-octadecanoyl-1-(3-phenylpropionyl)pyrrol-2-yl]propionic acid **13**

The mixture of **12** (33 mg, 0.05 mmol), palladium on carbon (10%) (15 mg) and ethyl acetate (5 ml) was stirred in a ground round-bottom flask with stopcock-equipment. A ground cone with stopcock attached with a balloon filled with hydrogen was put on the flask. While the stopcock of the flask was open the hydrogen was allowed to stream through the flask for some seconds. Than the stopcock of the cone and immediately after that the stopcock of the flask were closed avoiding that the pressure of the hydrogen in the flask exceeded atmospheric pressure. After 1 h kieselguhr was added and the reaction mixture was filtered. The solvent was carefully evaporated under reduced pressure at a bath temp. of 25–30 °C yielding **13** (21 mg, 74 %) as oil. ^{1}H-NMR: δ (ppm) = 0.88 (t, J = 7 Hz, 3H, CH_3), 1.14-1.37 [m, 28H, $(CH_2)_{14}$], 1.64 (quint, J = 7 Hz, 2H, CH_2CH_2CO), 2.11 (s, 3H, PyrCH$_3$), 2.42 (s, 3H, PyrCH$_3$), 2.51 (t, J = 8 Hz, 2H, CH_2), 2.68 (t, J = 8 Hz, 2H, CH_2), 2.85 (t, J = 8 Hz, 2H, CH_2), 3.06-3.15 (m, 4H, CH_2CH_2), 7.19-7.31 (m, 5H, aromat.H).– Anal. ($C_{36}H_{55}NO_4$) C, H, N.

3-(3,5-Dimethyl-4-octadecanoylpyrrol-2-yl)propionic acid **14**

The mixture of **5** (90 mg, 0.2 mmol), EtOH (15 ml) and 10% aqueous KOH (5 ml) was refluxed for 15 min. The cooled reaction mixture was diluted with water, acidified with dilute HCl and extracted with Et_2O. The organic layer was washed with dilute HCl, dried (Na_2SO_4) and evaporated. Precipitation from petroleum ether yielded **14** (55 mg, 63 %) as solid. Mp 108–109 °C.– ^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.88 (t, J = 7 Hz, 3H, CH_3), 1.14–1.44 [m, 28H, $(CH_2)_{14}$], 1.67 (quint, J = 7 Hz, 2H, CH_2CH_2COPyr), 2.19 (s, 3H, PyrCH$_3$), 2.45 (s, 3H, PyrCH$_3$), 2.62 (t, J = 7 Hz, 2H, CH_2), 2.68 (t, J = 7 Hz, 2H, CH_2COPyr), 2.80 (t, J = 7 Hz, 2H, CH_2), 8.40 (s, 1H, NH).– Anal. ($C_{27}H_{47}NO_3$) C, H, N.

Methyl 3-(1,3,5-trimethyl-4-octadecanoylpyrrol-2-yl)propionate **15**

The mixture of **5** (170 mg, 0.38 mmol), methyl *p*-toluenesulfonate (78 mg, 0.42 mmol), tetrabutylammonium bromide (12 mg, 0.038 mmol), Et_2O (5 ml), and powdered NaOH (60 mg, 1.5 mmol) was stirred at room temp. for 32 h. The reaction mixture was diluted with water and extracted with Et_2O. The organic layer was dried (Na_2SO_4) and evaporated. The residue was chromatographed on silica gel with petroleum ether-ethyl acetate (1. 8.5+1.5, 2. 8+2) yielding **15** (160 mg, 91 %) as solid. Mp 54–55 °C.– ^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.88 (t, J = 7 Hz, 3H, CH_3), 1.11–1.41 [m, 28H, $(CH_2)_{14}$], 1.66 (quint, J = 7 Hz, 2H, CH_2CH_2COPyr), 2.20 (s, 3H, Pyr-CH$_3$), 2.44 (t, J = 8 Hz, 2H, CH_2), 2.46 (s, 3H, PyrCH$_3$), 2.69 (t, J = 7 Hz, 2H, CH_2COPyr), 2.90 (t, J = 8 Hz, 2H, CH_2), 3.43 (s, 3H, NCH_3), 3.69 (s, 3H, OCH_3).– MS (EI): *m/z* (%) = 461 (19)[M^+], 388 (28), 222 (100), 184 (34).

3-(1,3,5-Trimethyl-4-octadecanoylpyrrol-2-yl)propionic acid **16**

The mixture of **15** (90 mg, 0.2 mmol), EtOH (15 ml) and 10% aqueous KOH (5 ml) was refluxed for 15 min. The cooled reaction mixture was diluted with water, acidified with dilute HCl and extracted with Et_2O. The organic layer was washed with dilute HCl, dried (Na_2SO_4) and evaporated. Precipitation from MeOH yielded **16** (65 mg, 73 %) as solid. Mp 104–105 °C.–

^{1}H NMR ($CDCl_3$): δ (ppm) = 0.88 (t, J = 7 Hz, 3H, -CH_3), 1.12–1.38 [m, 28H, $(CH_2)_{14}$], 1.66 (quint, J = 7 Hz, 2H, CH_2CH_2COPyr), 2.21 (s, 3H, $PyrCH_3$), 2.46 (s, 3H, $PyrCH_3$), 2.50 (t, J = 8 Hz, 2H, CH_2), 2.70 (t, J = 7 Hz, 2H, CH_2COPyr), 2.92 (t, J = 8 Hz, 2H, CH_2), 3.44 (s, 3H, N-CH_3).– Anal. ($C_{28}H_{49}NO_3$) C, H, N.

Biochemistry

cPLA$_2$-Inhibition

Inhibition of cPLA$_2$ was determined by measuring calcium ionophore A23187- or TPA-induced arachidonic acid release from bovine platelets with HPLC/UV-detection as previously described [29,31]. Briefly, to a solution of 5,8,11,14-eicosatetraynoic acid (ETYA), which inhibits formation of arachidonic acid metabolites in platelets, the test compound solution or the solvent was added followed by the platelet suspension and a solution of calcium chloride at 37 °C. Then cPLA$_2$ was activated by calcium ionophore A23187 or TPA. After termination of the enzyme reaction the produced arachidonic acid was cleaned up by solid-phase extraction and quantified with HPLC/UV-detection at 200 nm. The compounds were dissolved in DMSO.

Cell Lysis

Cell lysis was measured by turbidimetry as previously described[33]. Briefly, to a solution of ETYA the test compound solution or the solvent was added followed by the platelet suspension and a solution of calcium chloride at 37 °C. After dilution with phosphate buffered saline the absorbance of the cell suspensions was measured at 800 nm. Cell lysis led to a decrease of absorbance. The compounds were dissolved in DMSO.

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