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Structure–activity relationship studies on (4-acylpyrrol-2-yl)alkanoic acids as inhibitors of the cytosolic phospholipase A_2: Variation of the alkanoic acid substituent, the acyl chain and the position of the pyrrole nitrogen

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Summary — (4-Acylpyrrol-2-yl)alkanoic acid derivatives were prepared and evaluated for their ability to inhibit the cytosolic phospholipase A_2 of intact bovine platelets. To define the structural requirements for enzyme inhibition, the alkanoic acid group, the acyl residue and the position of the pyrrole nitrogen relative to the pyrrole substituents were varied systematically. Inhibition of $cPLA_2$ was best by compounds containing a free acetic acid or propionic acid group and an acyl chain of 12 or more carbons. The position of the pyrrole nitrogen did not influence the activity significantly. One of the most potent of the $cPLA_2$ inhibitors synthesized was (1,3,5-trimethyl-4-octadecanoylpyrrol-2-yl)acetic acid (IC_{50}: 10 μM).

cytosolic phospholipase A_2 / (4-acylpyrrol-2-yl)alkanoic acid / inhibitor / structure–activity relationship

Introduction

Phospholipases A_2 (PLA_2) are a class of enzymes which catalyze the hydrolysis of membrane phospholipids at the *sn*-2 position to release fatty acids and lysophospholipids. When the fatty acid is arachidonic acid, subsequent metabolism by cyclooxygenase and 5-lipoxygenase leads to the formation of lipid mediators such as prostaglandins and leukotrienes, which are believed to play a major role in the pathogenesis of inflammation and asthma, respectively [1, 2]. The other product 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].

One problem associated with the in vitro search for anti-inflammatory and anti-asthmatic PLA_2 inhibitors is the selection of the appropriate enzyme, because structurally different PLA_2 enzymes are present in the organism. In the synovial fluid of arthritic joints [5], and in the serum of patients with acute pancreatitis [6] and septic shock [7], high levels of the human non-pancreatic secretory PLA_2 (type II $sPLA_2$) [8] have been observed. This enzyme was therefore long regarded as the key control point of the arachidonic acid cascade. Many potent inhibitors of type II $sPLA_2$ have been developed, eg, the recently launched indole-3-acetamides and -glyoxamides [9] and the thielocin B3 derivatives [10]. However, inhibitors of this PLA_2 were effective only in some of the standard in vivo models of inflammation [11] and did not reduce arachidonic acid release in arthritic tissue [12]. Furthermore, transgenic mice which overexpressed the enzyme did not show signs of a systemic inflammation [13].

In 1990, the isolation of a higher-molecular-weight (85 kDa) cytosolic PLA_2 ($cPLA_2$) [14–17] from several cell types was reported; evidence has since accumulated that this enzyme controls the biosynthesis of the lipid mediators mentioned above [11, 12, 18–27]. So, for example, this enzyme selectively cleaves phospholipids containing arachidonic acid in *sn*-2 position contrary to the $sPLA_2$, which do not show any degree of selectivity for the hydrolysis of arachidonic acid at the scissile ester position of the substrate [27]. Moreover, inhibitors of $cPLA_2$ proved to be active in acute and chronic models of inflammation [11] and suppressed arachidonic acid release in arthritic tissue [12]. Such inhibitors might therefore serve as useful therapeutics for inflammatory diseases and asthma.

Despite several $cPLA_2$ inhibitors have been discovered, eg, (*S*)-*N*-hexadecylpyrrolidine-2-carboxamide (Wy-48,489) **1** [28–30] and arachidonyl trifluoromethyl ketone ($AACOCF_3$) **2** [31] (fig 1), no compound with clinical potential has emerged. Furthermore, only a few structure–activity relationship investigations concerning such inhibitors have been published to date [30, 32].

Recently we found that the 3-(4-octadecanoylpyrrol-2-yl)propionic acids **3** and **4** (fig 1) are inhibitors of $cPLA_2$ [33]. We now investigated the relationships between the inhibitory activity against $cPLA_2$ and the chemical structure of the alkanoic acid substituent in position 2 and of the acyl side chain in position 4 of the pyrroles. We further varied the position of the pyrrole nitrogen relative to the alkanoic acid group since the position of the pyrrole nitrogen in a pyrrole containing agent can have a strong influence on its biological activity. So structure–activity relationship studies on the cyclooxygenase-1 and the 5-lipoxygenase inhibitory potency of diphenylpyrrolizinylacetic acids had shown that, eg, the pyrrole derivative **5a** inhibited the cyclooxygenase-1 about 10 times more effective than its isomer **5b** [34, 35].

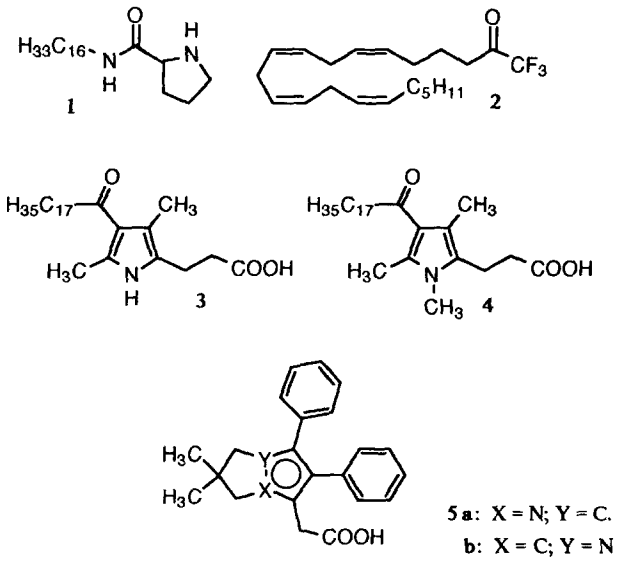


Fig 1.

Chemistry

The 3,5-dimethyl-4-octadecanoylpyrrole with a carboxylic acid moiety in position 2 of the pyrrole **6** (fig 2) was obtained by hydrolyzing its known ethyl ester [36]. For preparation of the homologous *N*-methyl derivative **7**, the ethyl ester of **6** was methylated with methyl *p*-toluenesulfonate prior to saponification.

The acetic acids **9** and **11** could be prepared by reacting 2,4-dimethyl-3-octadecanoylpyrrole **8** [33] and its 1-methylated derivative **10**, respectively, with ethyl diazoacetate in the presence of copper powder [37] followed by KOH hydrolysis of the ester intermediates (scheme 1).

The synthesis of the 4-(pyrrol-2-yl)butyric acid derivatives **15** and **16** started from 2,4-dimethylpyrrole **12** [38] (scheme 2). This was acylated in position 2 by a Friedel–Crafts reaction with methyl succinyl chloride [39] to form the methyl 4-oxobutyrate **13**. Reduction of the carbonyl function with $NaBH_4/BF_3$–Et_2O [40] and acylation with *N*,*N*-dimethyloctadecanamide/$POCl_3$ yielded **14**. Saponification or *N*-methylation and saponification led to the desired compounds **15** and **16**.

The synthesis of the methyl ester **17** (fig 2) has already been described [33]. The amide **18** was prepared by reacting **3** (fig 1) with *N*,*N*'-carbonyldiimidazole in CH_2Cl_2 followed by treatment with NH_3. The (*E*)-acrylic acid derivative **19** was afforded by hydrolyzing its ethyl ester [33]. In order to synthesize the 3,5-dimethylpyrrole-2-carboxylic acid with an octadecyl residue in position 4 (**20**) the carbonyl func-

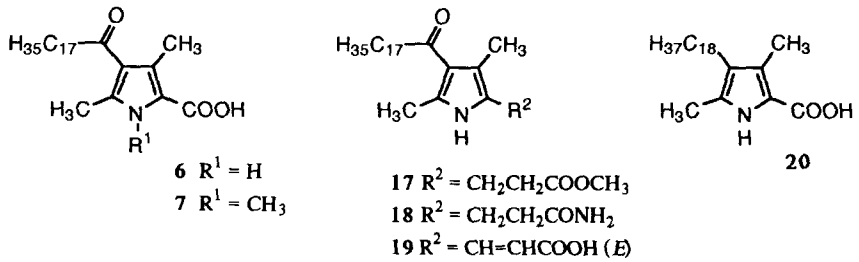
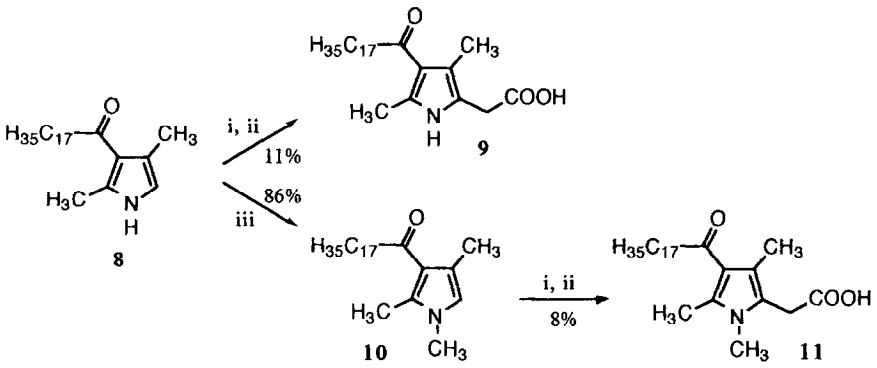
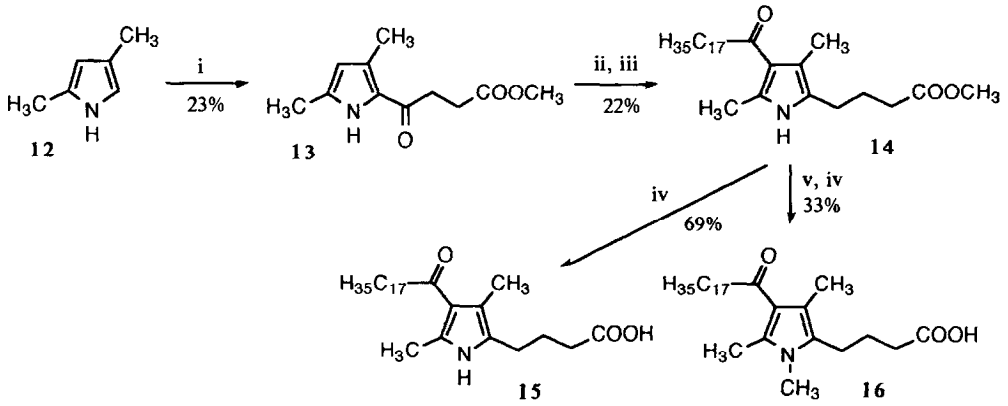


Fig 2.



Scheme 1. (i) Ethyl diazoacetate, Cu^0, toluene, 110–120 °C; (ii) aqueous KOH, EtOH; (iii) methyl *p*-toluenesulfonate, $(C_4H_9)_4N^+Br^-$, 50% aqueous NaOH, Et_2O.



Scheme 2. (i) Methyl succinyl chloride, $AlCl_3$, CH_2Cl_2; (ii) $NaBH_4$, BF_3–Et_2O, THF, methyl acetate, CH_2Cl_2; (iii) *N,N*-dimethyloctadecanamide, $POCl_3$, benzene; (iv) aqueous KOH, EtOH; (v) methyl *p*-toluenesulfonate, $(C_4H_9)_4N^+Br^-$, powdered NaOH, Et_2O, CH_2Cl_2.

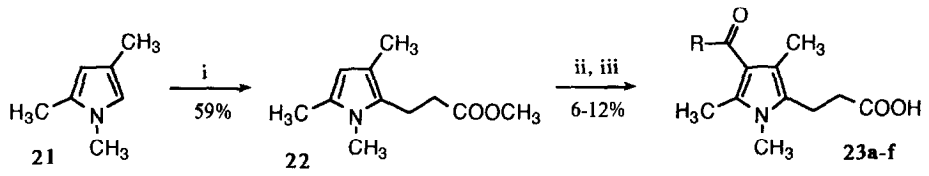
tion of the ethyl ester of **6** was reduced with $NaBH_4/BF_3$–Et_2O prior to ester hydrolysis.

3-(1,3,5-Trimethylpyrrol-2-yl)propionic acids with different 4-acyl chains (**23a–f**) were obtained starting from 1,2,4-trimethylpyrrole **21** [41]. First a methyl propionate chain was introduced in the α-position of the pyrrole nitrogen by reaction with methyl acrylate/ BF_3–Et_2O [42] to give **22**. From this the targets **23a–f** were yielded by Friedel–Crafts acylation and KOH hydrolysis (scheme 3).

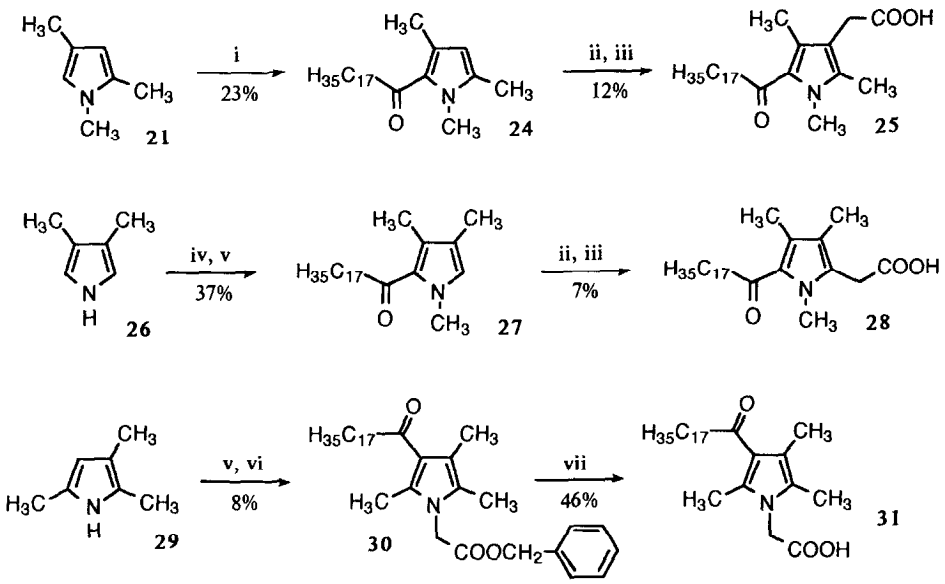
The isomers of compound **11** obtained by varying the position of the pyrrole nitrogen (**25**, **28**, **31**) were synthesized as shown in scheme 4. The acylation was accomplished by Friedel–Crafts or Vilsmeier reactions and the acetic acid functionality was introduced by copper-catalyzed coupling with ethyl diazoacetate or *tert*-BuOK–DMSO alkylation with benzyl bromoacetate followed by ester cleavage with aqueous base or Pd/C–hydrogen.

Pharmacology

The biological activity of the test compounds was evaluated by measuring the calcium ionophore A23187-induced arachidonic acid release from bovine platelets [43]. This assay detects inhibitors of $cPLA_2$ [20–22, 44, 45]. Since $cPLA_2$ inhibition is simulated



Scheme 3. (i) Methyl acrylate, BF_3–Et_2O, CH_2Cl_2; (ii) $C_nH_{2n+1}COCl$ (n = 6–9, 11, 15), $AlCl_3$, CH_2Cl_2; (iii) aqueous KOH, EtOH.



Scheme 4. (i) Octadecanoyl chloride, $AlCl_3$, CH_2Cl_2; (ii) ethyl diazoacetate, Cu^0, toluene, 110–120 °C; (iii) aqueous KOH, EtOH; (iv) methyl *p*-toluenesulfonate, $(C_4H_9)_4N^+Br^-$, powdered NaOH, Et_2O; (v) *N,N*-dimethyloctadecanamide, $POCl_3$, benzene; (vi) benzyl bromoacetate, *tert*-BuOK, DMSO; (vii) H_2, Pd/C, THF, EtOH.

when a substance leads to lysis of the platelets [46], furthermore, the cell lytic potency of each test compound was determined by turbidimetry.

Results and discussion

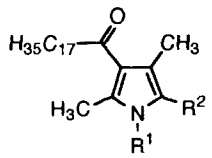
The reference compounds Wy-48,489 **1** and $AACOCF_3$ **2** inhibited the $cPLA_2$ of bovine platelets with an IC_{50} of 13 μM and 11 μM, respectively; for the pyrrole leads **3** and **4**, IC_{50}-values of 25 μM and 13 μM respectively have been evaluated [33] (table I).

The variation of the propionic acid side chain of the acylpyrroles **3** and **4** led to the following results (table I): Elongation by one carbon caused a decrease of activity; the *N*-unsubstituted butyric acid derivative **15** did not inhibit $cPLA_2$ at the highest concentration measured (33 μM), its *N*-methyl analogue **16** reduced arachidonic acid release only by 34% at 33 μM. The acetic acids **9** and **11** (IC_{50}: 15 μM and 10 μM, respectively) showed about the same inhibitory potency as the lead **4**. However, a further shortening of the alkanoic acid chain length led to compounds which were significantly less active than **4**; the IC_{50} of the *N*-unsubstituted pyrrole-2-carboxylic acid **6** ascended to 29 μM and the corresponding *N*-methylated compound **7** showed only a 20% inhibition of $cPLA_2$ at 33 μM.

Next, the effect of a derivatisation and a rigidisation of the propionic acid substituent was investigated. The methyl ester **17** and the amide derivative **18** of the propionic acid **3** did not inhibit $cPLA_2$ at 33 μM. Therefore, a free carboxylic acid moiety seems to be necessary for the activity of the compounds. The rigid (*E*)-acrylic acid **19** was also inactive, so the flexibility of the propionic acid group is required to achieve enzyme inhibition at a concentration of 33 μM or below.

To evaluate the role of the carbonyl function of the octadecanoyl residue regarding enzyme inhibition we intended to reduce the carbonyl of the acyl residue to a methylene group. Since it could be expected that such derivatives of the potent propionic acid **4** or acetic acid **11** would be very unstable because of the lack of any electron-withdrawing substituent at the pyrrole nucleus in such compounds [41], we decided to start this investigation from the less active 3,5-dimethyl-4-octadecanoylpyrrole-2-carboxylic acid **6**. The corresponding pyrrole with a 4-octadecyl residue **20**, which was stable because of the electron-withdrawing carboxylic acid group, showed no activity at 33 μM (table II). On the contrary, the relative inhibition of the enzyme by compound **6** was greater than

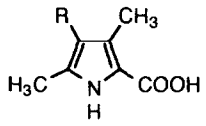
Table I. $cPLA_2$ inhibitory activity of (3,5-dimethyl-4-octadecanoylpyrrol-2-yl)alkanoic acids and some derivatives.



Compound	R^1	R^2	IC_{50} (μM)[a]
3	H	$(CH_2)_2COOH$	25 ± 3.5
4	CH_3	$(CH_2)_2COOH$	13 ± 2.3
6	H	COOH	29 ± 1.0
7	CH_3	COOH	> 33[b]
9	H	CH_2COOH	15 ± 3.0
11	CH_3	CH_2COOH	10 ± 2.1
15	H	$(CH_2)_3COOH$	NA[c]
16	CH_3	$(CH_2)_3COOH$	> 33[d]
17	H	$(CH_2)_2COOCH_3$	NA[c]
18	H	$(CH_2)_2CONH_2$	NA[c]
19	H	CH=CHCOOH (*E*)	NA[c]
Wy-48,489 (**1**)			13 ± 1.9
$AACOCF_3$ (**2**)			11 ± 2.4

[a]IC_{50} values are means ± SD, $n = 3$ (in case of **1**: $n = 4$, in case of **4**: $n = 5$); [b]20% inhibition at 33 μM; [c]NA: not active at 33 μM; [d]34% inhibition at 33 μM.

Table II. $cPLA_2$ inhibitory activity of 3,5-dimethylpyrrole-2-carboxylic acids with 4-octadecanoyl or 4-octadecyl residues.



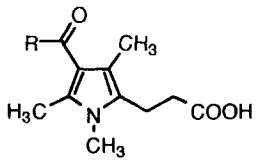
Compound	*R*	IC_{50} (μM)
6	$COC_{17}H_{35}$	29
20	$C_{18}H_{37}$	NA[a]

[a]NA: not active at 33 μM.

50% at 33 μM (IC_{50}: 29 μM). Thus the carbonyl function of the acyl residue exerts an influence on the pharmacological activity of the compounds.

Next, we wished to explore the effect of shortening the octadecanoyl chain on inhibitory potency. Replacement of the octadecanoyl residue of **4** with a hexadecanoyl and a dodecanoyl chain, respectively, did not change activity. For the compounds **4**, **23a** and **23b** an IC_{50} of 13 μM was evaluated in each case (table III). However, a further shortening of the acyl chain led to a decrease of inhibitory potency. While the decanoyl derivative **23c** was about 1.5 times less active (IC_{50}: 20 μM) compared to **4**, the IC_{50} of the nonanoyl compound **23d** and the octanoyl compound **23e** ascended to a value greater than 33 μM. The

Table III. $cPLA_2$ inhibitory activity of 3-(4-acyl-1,3,5-dimethylpyrrol-2-yl)propionic acids.



Compound	*R*	IC_{50} (μM)
4	$C_{17}H_{35}$	13
23a	$C_{15}H_{31}$	13
23b	$C_{11}H_{23}$	13
23c	C_9H_{19}	20
23d	C_8H_{17}	> 33[a]
23e	C_7H_{15}	> 33[b]
23f	C_6H_{13}	NA[c]

[a]40% inhibition at 33 μM; [b]18% inhibition at 33 μM; [c]NA: not active at 33 μM.

appropriate pyrrole with a heptanoyl residue **23f** even was inactive at 33 μM. Thus the length of the acyl chain in position 4 is of importance for inhibitory activity. Similar results were obtained when varying the acyl substituent of the $cPLA_2$ inhibitor 1-methyl-3-octadecanoylindole-2-carboxylic acid (paper submitted for publication): the activity of this compound also decreased when the chain length was shortened beyond 12 carbons. Since attempts to increase the potency of the indole-2-carboxylic acids by conformational restriction of the acyl chain failed, further variations of the acyl substituent of the pyrrolylalkanoic acids were not carried out.

Finally, we varied the position of the pyrrole nitrogen relative to the alkanoic acid and the acyl substituents. This exploration started from the acetic acid **11**. The isomeric acetic acids **25**, **28** and **31** were about as active as **11** (table IV). So contrary to the above-mentioned pyrrolizines **5a** and **5b**, the position of the pyrrole nitrogen is not important for the biological activity of the acylpyrrolylalkanoic acids.

Recently we have shown that $cPLA_2$ inhibition is faked when a substance leads to lysis of the platelets [46]. Therefore, we also measured the cell lytic potency of the test compounds by turbidimetry. With exception of the reference substance **2** (31% cell lysis at 33 μM) none of the tested compounds caused cell lysis even up to the concentration of 33 μM.

Although several inhibitors of $cPLA_2$ have been described in the literature [28–32], little is known about the way these substances affect enzyme activity. Different mechanisms of action are possible: eg, a compound can incorporate into the phospholipid substrate assembly and cause a change in enzyme rate by perturbing the physicochemical properties of the phospholipid membrane, or it can act as allosteric inhibitor, or it can interact directly with the active site of the enzyme.

Since the most potent inhibitors found have a detergent-like structure, it might be that they are non-specific membrane or substrate modifiers, acting through their amphiphilic properties. However, indications that the pyrroles affect the activity of $cPLA_2$ more likely by a specific interaction with the enzyme than by a perturbation or disintegration of the cell membrane were given from the following results.

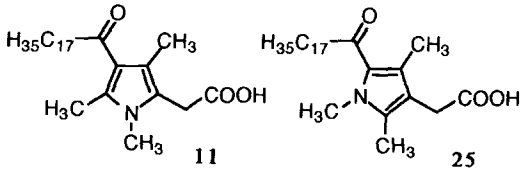
While the compounds **4**, **23a** and **23b** inhibited the activity of the $cPLA_2$ to the same extent (IC_{50}: 13 μM in each case), their cell lytic potency at 100 μM was significantly different in part.

While **4** did not cause cell lysis at 100 μM, **23a** and **23b** led to a cell lysis of 91% and 80%, respectively, at this concentration. Therefore, perturbation of the physicochemical properties of the cell membrane, which will lead to cell lysis at high concentrations, and the evaluated inhibition of A23187-induced arachidonic acid release are two distinct effects of the compounds. A reduced water solubility of **4** at 100 μM as reason for the absence of cell lysis could be excluded, since no precipitation was observed when adding phosphate buffered saline to the solution of **4** under test conditions.

In conclusion, we have shown that inhibition of $cPLA_2$ was best by pyrrole compounds which contained a free acetic acid or propionic acid group and an acyl chain of 12 or more carbons. The position of the pyrrole nitrogen relative to these substituents is not of great importance for the $cPLA_2$ inhibitory activity. Some of the obtained pyrroles are about as active as the reference compounds **1** and **2**, which are counted among the most active $cPLA_2$ inhibitors known today

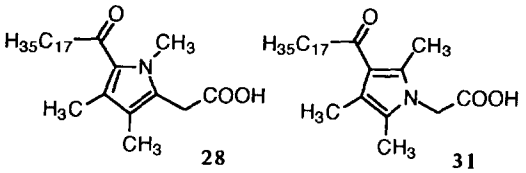
The forthcoming publication will report the results of studies on structure–activity relationships in terms of the structure of the substituents in position 1, 3 and 5 of the pyrroles.

Table IV. $cPLA_2$ inhibitory activity of isomeric (trimethyl-octadecanoylpyrrolyl)acetic acids.



11

25



28

31

Compound	*IC_{50} (μM)*
11	10
25	11
28	15
31	14

Experimental protocols

Chemical synthesis

All organic extracts were dried over Na_2SO_4. Melting points were determined in open capillary tubes with a Büchi melting point apparatus and are uncorrected. ^1H nuclear magnetic resonance spectra were recorded on a Jeol JNM-GX 400 spectrometer (400 MHz); chemical shifts (δ) are expressed in ppm, relative to internal tetramethylsilane. Mass spectra were obtained on a Varian CH7 apparatus; Electron beam ionisation at 70 eV (EI) was applied. Elemental analyses were determined

on a Heraeus CHN Rapid instrument and were within ±0.4% of theoretical values. Column chromatography was performed with Kieselgel 60 (70–230 mesh) silica gel (Merck).

The starting materials were obtained from commercial suppliers and used without further purification, or they were synthesized in the same or a similar manner as described in the literature cited. Reference compounds for the biological assays: arachidonyl trifluoromethyl ketone was purchased from Biomol (Hamburg); (*S*)-*N*-hexadecylpyrrolidine-2-carboxamide was synthesized by known procedures [32].

3,5-Dimethyl-4-octadecanoylpyrrole-2-carboxylic acid ***6***

The mixture of ethyl 3,5-dimethyl-4-octadecanoylpyrrole-2-carboxylate [36] (152 mg, 0.35 mmol), EtOH (15 mL) and 10% aqueous KOH (5 mL) was refluxed for 2 h, cooled, diluted with water, acidified with dilute HCl and extracted twice with $CHCl_3$. The organic phases were dried and the solvent was evaporated. The residue was dissolved in a small amount of warm CH_2Cl_2. Then the product was precipitated by adding petroleum ether. Yield 100 mg (70%). Mp 139–141 °C. ^{1}H-NMR ($CDCl_3$): δ = 0.88 (t, *J* = 7 Hz, 3H, CH_3), 1.12–1.44 [m, 28H, $(CH_2)_{14}$], 1.68 (quint, *J* = 7 Hz, 2H, CH_2CH_2CO), 2.53 (s, 3H, $PyrCH_3$), 2.62 (s, 3H, $PyrCH_3$), 2.73 (t, *J* = 7 Hz, 2H, CH_2CO), 8.97 (s, 1H, aromatic H). Anal $C_{25}H_{43}NO_3$ (C, H, N).

1,3,5-Trimethyl-4-octadecanoylpyrrole-2-carboxylic acid ***7***

The mixture of ethyl 3,5-dimethyl-4-octadecanoylpyrrole-2-carboxylate [36] (217 mg, 0.50 mmol), methyl *p*-toluenesulfonate (102 mg, 0.55 mmol), tetrabutylammonium bromide (16 mg, 0.05 mmol), Et_2O (10 mL), CH_2Cl_2 (2 mL) and powdered NaOH (80 mg, 2 mmol) was stirred at room temperature for 8 h. The reaction mixture was diluted with water and extracted twice with Et_2O. The organic layer was dried and evaporated. The residue was recrystallized from MeOH to give ethyl 1,3,5-trimethyl-4-octadecanoylpyrrole-2-carboxylate (yield 180 mg, 80%; mp 64–65 °C). An aliquot of this intermediate (157 mg, 0.35 mmol) was saponified using the same method as for the preparation of **6**. The product was precipitated from MeOH. Yield 116 mg (79%). Mp 100–102 °C. ^{1}H-NMR ($CDCl_3$): δ = 0.88 (t, *J* = 7 Hz, 3H, CH_3), 1.16–1.40 [m, 28H, $(CH_2)_{14}$], 1.67 (quint, *J* = 7 Hz, 2H, CH_2CH_2CO), 2.45 (s, 3H, $PyrCH_3$), 2.57 (s, 3H, $PyrCH_3$), 2.74 (t, *J* = 7 Hz, 2H, CH_2CO), 3.81 (s, 3H, NCH_3). Anal $C_{26}H_{45}NO_3$ (C, H, N).

(3,5-Dimethyl-4-octadecanoylpyrrol-2-yl)acetic acid ***9***

The solution of **8** [33] (145 mg, 0.4 mmol) in dry toluene (3 mL) was treated with the solution of ethyl diazoacetate (68 mg, 0.60 mmol) in dry toluene (1 mL) and powdered copper (about 0.4 g) in an oil bath at 115–120 °C until development of nitrogen ceased (about 10 min). The addition of ethyl diazoacetate and copper powder was repeated twice in the same way. The cooled reaction mixture was chromatographed on silica gel with petroleum ether–ethyl acetate (17 + 3). To the obtained ethyl ester of **9**, EtOH (15 mL) and 10% aqueous KOH (5 mL) were added and the resulting mixture was refluxed for 15 min, cooled, diluted with water, acidified with dilute HCl and extracted with Et_2O. The organic phase was washed with dilute HCl, dried and the product precipitated from Et_2O–petroleum ether. Yield 19 mg (11%). Mp 97–99 °C. ^{1}H-NMR ($CDCl_3$): δ = 0.88 (t, *J* = 7 Hz, 3H, CH_3), 1.16–1.39 [m, 28H, $(CH_2)_{14}$], 1.67 (quint, *J* = 7 Hz, 2H, CH_2CH_2CO), 2.20 (s, 3H, $PyrCH_3$), 2.49 (s, 3H, $PyrCH_3$), 2.69 (t, *J* = 7 Hz, 2H, CH_2CO), 3.61 (s, 2H, CH_2COOH), 8.44 (b, 1H, NH). Anal $C_{26}H_{45}NO_3$ (C, H, N).

(1,3,5-Trimethyl-4-octadecanoylpyrrol-2-yl)acetic acid ***11***

The mixture of **8** [33] (0.98 g, 2.7 mmol), methyl *p*-toluenesulfonate (0.56 g, 3 mmol), tetrabutylammonium bromide (0.17 g, 0.54 mmol), Et_2O (15 mL) and 50% aqueous NaOH (5 mL) was refluxed for 4 h with vigorous stirring. The reaction mixture was cooled and the organic phase separated. The aqueous layer was extracted twice with Et_2O and the combined organic phases were dried and evaporated down to some mL. After addition of MeOH 1,2,4-trimethyl-3-octadecanoylpyrrole **10** precipitated (yield 0.87 g, 86%; mp 56–58 °C). The solution of an aliquot of **10** (300 mg, 0.8 mmol) in dry toluene (3 mL) was treated with ethyl diazoacetate and saponified using a similar method as for the synthesis of **9**. The product **11** was precipitated from petroleum ether. Yield 29 mg (8%). Mp 98–99 °C. ^{1}H-NMR ([d_6]DMSO): δ = 0.86 (t, *J* = 7 Hz, 3H, CH_3), 1.12–1.34 [m, 28H, $(CH_2)_{14}$], 1.47–1.58 (m, 2H, CH_2CH_2CO), 2.10 (s, 3H, $PyrCH_3$), 2.39 (s, 3H, $PyrCH_3$), 2.62 (t, *J* = 7 Hz, 2H, CH_2CO), 3.38 (s, 3H, NCH_3), 3.56 (s, 2H, CH_2COOH), 12.38 (b, 1H, COOH). Anal $C_{27}H_{47}NO_3$ (C, H, N).

Methyl 4-(3,5-dimethylpyrrol-2-yl)-4-oxobutyrate ***13***

The solution of 2,4-dimethylpyrrole **12** [38] (500 mg, 5.3 mmol) was treated at –20 °C with the mixture of methyl succinyl chloride (750 mg, 5 mmol), $AlCl_3$ (693 mg, 5.2 mmol) and dry CH_2Cl_2 (5 mL). The resultant mixture was stirred for 1 h at –20 °C and then for 1 h at room temperature. After addition of water the mixture was extracted twice with CH_2Cl_2. The organic layers were washed with saturated aqueous $NaHCO_3$ solution, dried and concentrated. The residue was chromatographed on silica gel using CH_2Cl_2–petroleum ether–ethyl acetate (9 + 5 + 1) as eluent. The product was precipitated from petroleum ether. Yield 260 mg (23%). Mp 158–159 °C. ^{1}H-NMR ($CDCl_3$): δ (ppm) = 2.24 (s, 3H, $PyrCH_3$), 2.36 (s, 3H, $PyrCH_3$), 2.73 (t, *J* = 7 Hz, 2H, CH_2), 3.03 (t, *J* = 7 Hz, 2H, CH_2), 3.70 (s, 3H, OCH_3), 5.82 (s, 1H, aromatic H), 9.02 (s, 1H, aromatic H). MS: *m/z* (%) = 209 (32) [M$^+$], 178 (16), 122 (100), 94 (22).

Methyl 4-(3,5-dimethyl-4-octadecanoylpyrrol-2-yl)butyrate ***14***

The mixture of **13** (250 mg, 1.2 mmol), dry THF (3.5 mL), dry methyl acetate (5 mL), dry CH_2Cl_2 (5 mL), $NaBH_4$ (140 mg) and BF_3–Et_2O (0.75 mL) was stirred at room temperature for 1 h. After addition of MeOH (5 mL) the mixture was refluxed for 5 min, cooled, diluted with water and extracted twice with Et_2O. The organic layers were washed with saturated aqueous $NaHCO_3$ solution, dried and concentrated. The residue was dissolved in benzene (5 mL) and added to the refluxing solution of *N,N*-dimethyloctadecanamide (0.69 g, 2.2 mmol) and $POCl_3$ (230 mg, 1.5 mmol) in dry benzene (10 mL). The resulting solution was refluxed for 4 h. After addition of the solution of sodium acetate (2 g) in water (8 mL) the mixture was heated to reflux for further 15 min with vigorous stirring. The reaction mixture was cooled, diluted with water and extracted with Et_2O. The organic layer was washed with saturated aqueous $NaHCO_3$ solution, dried and concentrated. The residue was chromatographed on silica gel using petroleum ether–ethyl acetate (8.5 + 1.5) as eluent. The product was precipitated from MeOH. Yield 120 mg (22%). Mp. 73–74 °C. ^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.88 (t, *J* = 7 Hz, 3H, CH_3), 1.16–1.42 [m, 28H, $(CH_2)_{14}$], 1.61–1.74 (m, 2H, CH_2), 1.84 (quint, *J* = 7 Hz, 2H, CH_2), 2.17 (s, 3H, $PyrCH_3$), 2.32 (t, *J* = 7 Hz, 2H, CH_2), 2.47 (s, 3H, $PyrCH_3$), 2.54 (t, *J* = 7 Hz, 2H, CH_2), 2.68 (t, *J* = 7 Hz, 2H, CH_2), 3.68 (s, 3H, OCH_3), 8.08 (s, 1H, aromatic H). MS: *m/z* (%) = 461 (9) [M$^+$], 429 (14), 237 (24), 190 (100).

4-(3,5-Dimethyl-4-octadecanoylpyrrol-2-yl)butyric acid ***15***
14 (60 mg, 0.13 mmol) was saponified with KOH using a similar method as for the synthesis of **9**. The product was precipitated from MeOH. Yield 40 mg (69%). Mp 118–120 °C. ^{1}H-NMR ($CDCl_3$): δ = 0.88 (t, *J* = 7 Hz, 3H, CH_3), 1.14–1.40 [m, 28H, $(CH_2)_{14}$], 1.67 (quint, *J* = 7 Hz, 2H, CH_2), 1.87 (quint, *J* = 7 Hz, 2H, CH_2), 2.19 (s, 3H, PyrCH_3), 2.35 (t, *J* = 7 Hz, 2H, CH_2), 2.48 (s, 3H, PyrCH_3), 2.59 (t, *J* = 7 Hz, 2H, CH_2), 2.69 (t, *J* = 7 Hz, 2H, CH_2), 8.20 (s, 1H, aromatic H). Anal $C_{28}H_{49}NO_3$ (C, H, N).

4-(1,3,5-Trimethyl-4-octadecanoylpyrrol-2-yl)butyric acid ***16***
14 (46 mg, 0.1 mmol) was *N*-methylated applying a similar method as described for the synthesis of **7** (reaction time: 15 h). An aliquot (23 mg, 0.05 mmol) of the obtained methyl ester of **16** (yield 32 mg, 67%) was saponified with KOH using a similar method as for the synthesis of **9**. The product was recrystallized from MeOH–water. Yield 11 mg. Mp 72–73 °C. ^{1}H-NMR ($CDCl_3$): δ = 0.88 (t, *J* = 7 Hz, 3H, CH_3), 1.16–1.42 [m, 28H, $(CH_2)_{14}$], 1.67 (quint, *J* = 7 Hz, 2H, CH_2), 1.79 (quint, *J* = 7 Hz, 2H, CH_2), 2.19 (s, 3H, PyrCH_3), 2.39 (t, *J* = 7 Hz, 2H, CH_2), 2.46 (s, 3H, PyrCH_3), 2.61 (t, *J* = 7 Hz, 2H, CH_2), 2.70 (t, *J* = 7 Hz, 2H, CH_2), 3.42 (s, 3H, NCH_3). Anal $C_{29}H_{51}NO_3$ (C, H, N).

3-(3,5-Dimethyl-4-octadecanoylpyrrol-2-yl)propanamide ***18***
The solution of 3-(3,5-dimethyl-4-octadecanoylpyrrol-2-yl)-propionic acid **3** [33] (87 mg, 0.2 mmol) and *N,N'*-carbonyldiimidazole (146 mg, 0.9 mmol) in dry THF (5 mL) and dry CH_2Cl_2 (5 mL) was stirred for 1 h at room temperature. Then ammonia solution 25% (5 mL) was added at 0 °C and the mixture was stirred for 1 h at the same temperature. The mixture was diluted with water and extracted with Et_2O. The organic layer was washed with dilute NaOH, dried and concentrated. The product was precipitated from Et_2O–petroleum ether. Yield 30 mg (35%). Mp 162–164 °C. ^{1}H-NMR ($CDCl_3$): δ = 0.85 (t, *J* = 7 Hz, 3H, CH_3), 1.13–1.39 [m, 28H, $(CH_2)_{14}$], 1.67 (quint, *J* = 7 Hz, 2H, CH_2CH$_2$COPyr), 2.19 (s, 3H, PyrCH_3), 2.45 (s, 3H, PyrCH_3), 2.47–2.51 (m, 2H, CH_2), 2.67 (t, *J* = 7 Hz, 2H, CH_2COPyr), 2.79–2.82 (m, 2H, CH_2), 5.38 (d, *J* = 30 Hz, 2H, $CONH_2$), 8.95 (s, 1H, aromatic H). Anal $C_{27}H_{48}N_2O_2$ (C, H, N).

(E)-3-(3,5-Dimethyl-4-octadecanoylpyrrol-2-yl)acrylic acid ***19***
The synthesis started from ethyl 3-(3,5-dimethyl-4-octadecanoylpyrrol-2-yl)acrylate [33] (92 mg, 0.2 mmol) using a similar method as for the synthesis of **6**. The product precipitated from diisopropyl ether. Yield 37 mg (43%). Mp 145–146 °C. ^{1}H-NMR ([d_6]DMSO): δ = 0.85 (t, *J* = 7 Hz, 3H, CH_3), 1.16–1.35 [m, 28H, $(CH_2)_{14}$], 1.49–1.60 (m, 2H, CH_2CH$_2$CO), 2.26 (s, 3H, PyrCH_3), 2.45 (s, 3H, PyrCH_3), 2.66 (t, *J* = 7 Hz, 2H, CH_2CO), 6.12 (d, *J* = 15 Hz, 1H, CHCOOH), 7.40 (d, *J* = 15 Hz, 1H, C*H*CHCOOH), 11.52 (s, 1H), 11.99 (s, 1H). Anal $C_{27}H_{45}NO_3$ (C, H, N).

3,5-Dimethyl-4-octadecylpyrrole-2-carboxylic acid ***20***
The mixture of ethyl 3,5-dimethyl-4-octadecanoylpyrrole-2-carboxylate [36] (152 mg, 0.35 mmol), dry THF (3.5 mL), dry methyl acetate (5 mL), $NaBH_4$ (70 mg) and BF_3–Et_2O (0.35 mL) was stirred at room temperature for 1 h. After addition of MeOH (5 mL) and water (5 mL) the mixture was stirred for further 15 min. Then it was diluted with water and extracted with Et_2O. The organic layer was dried and concentrated. The intermediate was saponified with KOH using a similar method as for the synthesis of **9** (reaction time: 5 h). The product was purified by silica gel chromatography (eluent: (1) petroleum ether–ethyl acetate 8 + 2, (2) petroleum ether–ethyl acetate–acetic acid 8 + 2 + 0.1) and precipitated from petroleum ether. Yield 31 mg (23%). Mp 85–87 °C. ^{1}H-NMR ($CDCl_3$): δ = 0.88 (t, *J* = 7 Hz, 3H, CH_3), 1.17–1.36 [m, 30H, $(CH_2)_{15}$], 1.36–1.45 (m, 2H, CH_2CH$_2$Pyr), 2.20 (s, 3H, PyrCH_3), 2.28 (s, 3H, PyrCH_3), 2.34 (t, *J* = 7 Hz, 2H, CH_2Pyr), 8.59 (br, 1H, NH). Anal $C_{25}H_{45}NO_2$ (C, H, N).

General procedure for the synthesis of 3-(4-acyl-1,3,5-trimethylpyrrol-2-yl)propionic acids ***23a–f***
The solution of 1,2,4-trimethylpyrrole **21** [40] (3.1 g, 28 mmol) and methyl acrylate (2.5 mL) in dry CH_2Cl_2 (40 mL) was treated dropwise with BF_3–Et_2O (1.7 mL) at 0 °C. The mixture was then stirred for 15 min at the same temperature, diluted with water and extracted twice with Et_2O. The organic phases were dried and the solvent evaporated. Purification by silica gel chromatography eluting with petroleum ether–ethyl acetate (9 + 1) gave methyl 3-(1,3,5-trimethylpyrrol-2-yl)propionate **22** as oil (yield 3.2 g, 59%). To the solution of **22** (293 mg, 1.5 mmol) and the appropriate carboxylic acid chloride (1.7 mmol) in dry CH_2Cl_2 (10 mL) was added $AlCl_3$ (253 mg, 1.9 mmol) in several portions at 0 °C. Then the mixture was stirred for 30 min at the same temperature. After addition of water and NaCl the mixture was extracted with Et_2O–CH_2Cl_2 (3 + 1). The organic layer was washed with saturated aqueous $NaHCO_3$ solution, dried and concentrated. The residue was chromatographed on silica gel using petroleum ether–ethyl acetate (1) 9 + 1, (2) 7 + 3 as eluent. An aliquot (0.20–0.25 mmol) of the obtained methyl ester of **23a–f** (yield 16–21%) was saponified with KOH using a similar method as for the synthesis of **9**. The product was precipitated from petroleum ether. Yield 29–73%.

3-(4-Dodecanoyl-1,3,5-trimethylpyrrol-2-yl)propionic acid ***23b***
Analytical data for a selected compound: mp 104–105 °C. ^{1}H-NMR ($CDCl_3$): δ = 0.88 (t, *J* = 7 Hz, 3H, CH_3), 1.15–1.40 [m, 16H, $(CH_2)_8$], 1.67 (quint, *J* = 7 Hz, 2H, CH_2), 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_2), 2.92 (t, *J* = 8 Hz, 2H, CH_2), 3.44 (s, 3H, NCH_3). Anal $C_{22}H_{37}NO_3$ (C, H, N).

(1,2,4-Trimethyl-5-octadecanoylpyrrol-3-yl)acetic acid ***25***
To the solution of **21** [40] (328 mg, 3 mmol) and octadecanoyl chloride (1.7 mmol) in dry CH_2Cl_2 (20 mL) was added $AlCl_3$ (480 mg, 3.6 mmol) in several portions at 0 °C. Then the mixture was stirred for 15 min at the same temperature. After addition of water the mixture was extracted with Et_2O. The organic layer was washed with saturated aqueous $NaHCO_3$ solution, dried and concentrated. The residue was chromatographed on silica gel using petroleum ether–ethyl acetate (19 + 1) as eluent and 1,3,5-trimethyl-2-octadecanoylpyrrole **24** was precipitated from MeOH (yield 261 mg, 23%; mp 52–54 °C). Starting from this intermediate (188 mg, 0.5 mmol) **25** was prepared using a similar method as for the synthesis of **9**. Yield 26 mg (12%). Mp 108–110 °C. ^{1}H-NMR ($CDCl_3$): δ = 0.88 (t, *J* = 7 Hz, 3H, CH_3), 1.16–1.42 [m, 28H, $(CH_2)_{14}$], 1.68 (quint, *J* = 7 Hz, 2H, CH_2), 2.19 (s, 3H, PyrCH_3), 2.30 (s, 3H, PyrCH_3), 2.71 (t, *J* = 7 Hz, 2H, CH_2CO), 3.46 (s, 2H, CH_2COOH), 3.77 (s, 3H, NCH_3). Anal $C_{27}H_{47}NO_3$ (C, H, N).

(1,3,4-Trimethyl-5-octadecanoylpyrrol-2-yl)acetic acid ***28***
The mixture of 3,4-dimethylpyrrole **26** [47] (0.34 g, 3.6 mmol), methyl *p*-toluenesulfonate (0.80 g, 4.3 mmol), tetrabutylammonium bromide (116 mg, 0.36 mmol), Et_2O (20 mL), powdered NaOH (600 mg, 15 mmol) and 4 drops of water was stirred at room temperature for 4 h and then filtered. The filter

cake was washed with CH_2Cl_2, the filtrates were dried and concentrated. The obtained intermediate was acylated with *N,N*-dimethyloctadecanamide/$POCl_3$ in an analogous way as described for the synthesis of **14** to give 1,3,4-trimethyl-2-octadecanoylpyrrole **27** (yield 0.5 g, 37%; mp 63–64 °C). Starting from this intermediate (263 mg, 0.7 mmol) **28** was prepared using a similar method as for the synthesis of **9**. Yield 20 mg (7%). Mp 96–98 °C. ^{1}H-NMR ($CDCl_3$): δ = 0.88 (t, *J* = 7 Hz, 3H, CH_3), 1.16–1.42 [m, 28H, $(CH_2)_{14}$], 1.68 (quint, *J* = 7 Hz, 2H, CH_2), 1.98 (s, 3H, $PyrCH_3$), 2.28 (s, 3H, $PyrCH_3$), 2.72 (t, *J* = 7 Hz, 2H, CH_2CO), 3.66 (s, 2H, CH_2COOH), 3.79 (s, 3H, NCH_3). Anal $C_{27}H_{47}NO_3$ (C, H, N).

*(2,3,5-Trimethyl-4-octadecanoylpyrrol-1-yl)acetic acid **31***

2,3,5-Trimethylpyrrole **29** [48] (0.55 g, 5 mmol) was acylated with *N,N*-dimethyloctadecanamide/$POCl_3$ in an analogous way as described for the synthesis of **14** to give 2,3,5-trimethyl-4-octadecanoylpyrrole (yield 1.0 g). To the intermediate *tert*-BuOK (335 mg, 3 mmol), benzyl bromoacetate (618 mg, 2.7 mmol) and dry DMSO (10 mL) were added and the mixture was heated with stirring in an oil bath at 120 °C for 15 min. The mixture was cooled, diluted with water and extracted three times with CH_2Cl_2. The organic phases were dried and concentrated. The residue was chromatographed on silica gel using petroleum ether–ethyl acetate (12 + 1) as eluent and benzyl (2,3,5-trimethyl-4-octadecanoylpyrrol-1-yl)acetate **30** was precipitated from MeOH (yield 219 mg, 8%; mp 61–63 °C). A solution of **30** (79 mg, 0.15 mmol) in a mixture of THF (10 mL) and EtOH (10 mL) was then treated with a catalytic amount of 10% Pd/C and the mixture hydrogenated at atmospheric pressure for 1 h. After addition of kieselguhr the mixture was filtered, the solvent evaporated and the product precipitated from petroleum ether. Yield 30 mg (46%). Mp 82–85 °C. ^{1}H-NMR ($CDCl_3$): δ = 0.88 (t, *J* = 7 Hz, 3H, CH_3), 1.16–1.42 [m, 28H, $(CH_2)_{14}$], 1.66 (quint, *J* = 7 Hz, 2H, CH_2), 2.10 (s, 3H, $PyrCH_3$), 2.19 (s, 3H, $PyrCH_3$), 2.43 (s, 3H, $PyrCH_3$), 2.71 (t, *J* = 7 Hz, 2H, CH_2CO), 4.57 (s, 2H, NCH_2CO). Anal $C_{27}H_{47}NO_3$ (C, H, N).

Biological evaluation

$cPLA_2$-Inhibition

Inhibition of $cPLA_2$ was determined by measuring calcium ionophore A23187-induced arachidonic acid release from bovine platelets with HPLC/UV-detection as previously described [43]. Briefly, to a solution of 5,8,11,14-eicosatetraynoic acid (ETYA), which inhibits formation of arachidonic acid metabolites in platelets, was added the test compound solution or the solvent (in the case of the control test) followed by the platelet suspension and a solution of calcium chloride at 37 °C. Then $cPLA_2$ was activated by calcium ionophore A23187. 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 test compounds were dissolved in DMSO, if necessary with heating. The IC_{50} values (50% inhibitory concentration) were determined graphically and are the means of at least two independent experiments. For the lead **4** the evaluated IC_{50} values lie in the range 11–16 μM (mean ± SD: 13 ± 2.3 μM, *n* = 5). Compound **4** was included as reference control in each experiment; at a concentration of 10 μM inhibition of arachidonic acid release by **4** ranged from 30% to 48% (mean ± SD: 39 ± 6%, *n* = 15). The compounds were tested up to a maximum concentration of 33 μM. 'IC_{50} > 33 μM' means that a substance was active at 33 μM, but the relative enzyme inhibition was below 50%; 'not active at 33 μM' indicates that the inhibition was 0% at this concentration. The enzyme reactions were performed within 36 h after isolation of the platelets. The platelets were stored at +4 °C.

Cell lysis

Cell lysis was measured by turbidimetry as previously described [46]. Briefly, to a solution of ETYA was added the test compound solution or the solvent (in the case of the control test) 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 test compounds were dissolved in DMSO, if necessary with heating. The cell lysis was determined within 36 h after isolation of the platelets. The platelets were stored at +4 °C.

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