Synthesis, Biological Evaluation, and Structure−Activity Relationships of 3-Acylindole-2-carboxylic Acids as Inhibitors of the Cytosolic Phospholipase A_2

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3-Acylindole-2-carboxylic 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 carboxylic acid group, the acyl residue, and the moiety in position 1 were systematically modified. Furthermore, different substituents were introduced into the phenyl part of the indole. Replacement of the carboxylic acid group in position 2 of the indole with an acetic or propionic acid substituent led to a decrease of inhibitory potency. Enzyme inhibition was optimal when the acyl residue in position 3 had a length of 12 or more carbons. Conformational restriction of the acyl residue did not influence activity. Introduction of alkyl chains at position 1 of the indole with 8 or more carbons resulted in a loss of activity. However, replacing the ω-methyl group of such compounds with a carboxylic acid moiety was found to increase inhibitory potency significantly. Among the tested indole derivatives, 1-[2-(4-carboxyphenoxy)ethyl]-3-dodecanoylindole-2-carboxylic acid (**29b**) had the highest potency. With an IC_{50} of 0.5 μM it was about 20-fold more active than the standard $cPLA_2$ inhibitor arachidonyl trifluoromethyl ketone (IC_{50}: 11 μM).

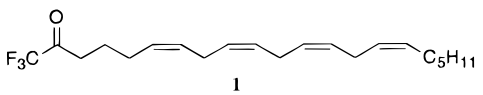
Introduction

Phospholipases A_2 (PLA_2) 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 enzymes 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]

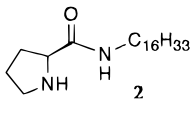
One problem associated with the *in vitro* search for antiinflammatory PLA_2 inhibitors is the selection of the appropriate enzyme, because structurally different PLA_2 enzymes are present in the organism. In the synovial fluid of arthritic joints[5] and in the serum of patients with severe acute pancreatitis,[6] septic shock,[7] and multiple injuries,[8] high levels of the human nonpancreatic secretory PLA_2 (type II $sPLA_2$)[9] 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, *e.g.* the recently published indole-3-acetamides and -glyoxamides[10] and the thielocin B3 derivatives.[11] However, inhibitors of this PLA_2 were effective only in some of the standard *in vivo* models of inflammation[12] and did not reduce arachidonic acid release in arthritic tissue.[13] Furthermore, transgenic mice which overexpressed the enzyme did not show signs of a systemic inflammation.[14]

In 1990, the isolation of a higher-molecular-weight (85 kDa) cytosolic PLA_2 ($cPLA_2$)[15−18] from several cell types was reported; evidence has since accumulated that this enzyme controls the biosynthesis of the lipid mediators mentioned above.[19−26] So, for example, this enzyme selectively cleaves phospholipids containing arachidonic acid in the *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.[27] Moreover, inhibitors of $cPLA_2$ proved to be active in acute and chronic models of inflammation[12] and suppressed arachidonic acid release in arthritic tissue.[13] Such inhibitors might therefore serve as useful therapeutics for inflammatory diseases and asthma. Possibly the antiinflammatory and antiasthmatic effectiveness 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 cytokines.[28−31]

Despite the fact that several inhibitors of $cPLA_2$ have been discovered, *e.g.* arachidonyl trifluoromethyl ketone ($AACOCF_3$)[32] (**1**) and *(S)*-*N*-hexadecylpyrrolidine-2-carboxamide (Wy-48,489)[33−35] (**2**), no compound has been reported to be undergoing clinical development. Moreover, only a few structure−activity relationship investigations relating to those enzyme inhibitors have been published to date.



1



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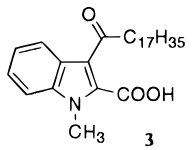

3

Recently we reported that 1-methyl-3-octadecanoylindole-2-carboxylic acid (**3**) is an inhibitor of $cPLA_2$.[36] In this paper we describe the synthesis, biological activity,

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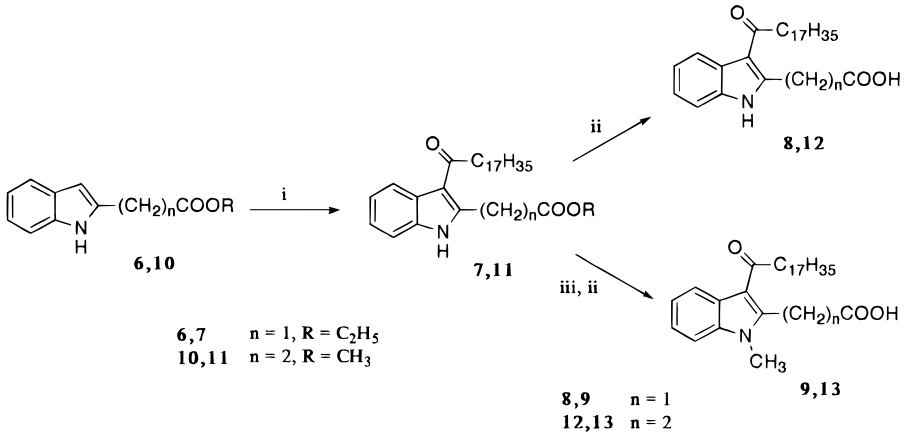
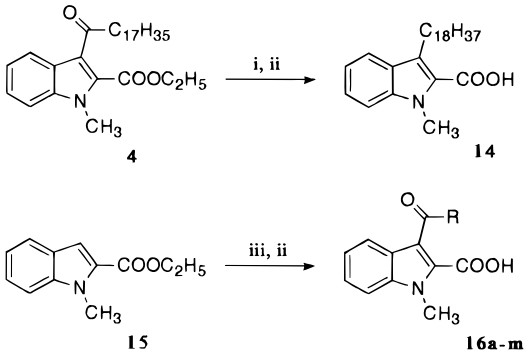
Scheme 1



[a] (i) *N*,*N*-Dimethyloctadecanamide, $POCl_3$, benzene; (ii) aqueous KOH, EtOH; (iii) methyl *p*-toluenesulfonate, $(C_4H_9)_4N^+Br^-$, powdered NaOH, Et_2O, CH_2Cl_2.

Scheme 2



[a] (i) $NaBH_4$, $BF_3{\cdot}Et_2O$, THF, methyl acetate; (ii) aqueous KOH, EtOH; (iii) carboxylic acid, trifluoroacetic anhydride, polyphosphoric acid, CH_2Cl_2.

and structure−activity relationships of a series of compounds derived from this lead.

Chemistry

The synthesis of 3-octadecanoylindoles with acetic acid and propionic acid side chains in position 2 started from ethyl (indol-2-yl)acetate (**6**) and methyl 3-(indol-3-yl)propionate (**10**), respectively (Scheme 1). The introduction of the octadecanoyl group in position 3 was accomplished by reaction with *N*,*N*-dimethyloctadecanamide/$POCl_3$ in benzene. Saponification of the intermediates **7** and **11** with KOH in ethanol yielded the desired test compounds **8** and **12**. The analogous *N*-methylindole derivatives **9** and **13** were afforded by treating **7** and **11**, respectively, with methyl *p*-toluenesulfonate in a phase transfer reaction prior to KOH hydrolysis.

The 1-methylindole-2-carboxylic acid with an octadecyl residue in position 3 (**14**) was prepared by reducing the carbonyl function of ethyl 1-methyl-3-octadecanoylindole-2-carboxylate[36] (**4**) upon treatment with $NaBH_4$/$BF_3{\cdot}Et_2O$ followed by KOH hydrolysis of the resulting intermediate (Scheme 2).

The 1-methylindole-2-carboxylic acids with varying 3-acyl chains (**16a**−**m**) were synthesized following a procedure described by Murakami et al. (Scheme 2).[37] Ethyl 1-methylindole-2-carboxylate (**15**) was acylated in position 3 by reaction with the appropriate carboxylic acid in CH_2Cl_2 in the presence of trifluoroacetic anhydride and polyphosphoric acid. Hydrolysis of the ester intermediates gave the desired test compounds **16a**−**m**.

Similarly, 1-methyl-3-dodecanoylindole-2-carboxylic acids with different substituents in the phenyl part of the indole were prepared starting from the appropriately substituted ethyl indole-2-carboxylates **17a**−**d**,**f**,**g** or ethyl 5-acetoxyindole-2-carboxylate (**17e**). Methylation in position 1 with methyl *p*-toluenesulfonate followed by acylation in position 3 with dodecanoic acid and saponification of the resulting ethyl 3-dodecanoyl-1-methylindole-2-carboxylates yielded the target compounds **18a**−**g** (Scheme 3).

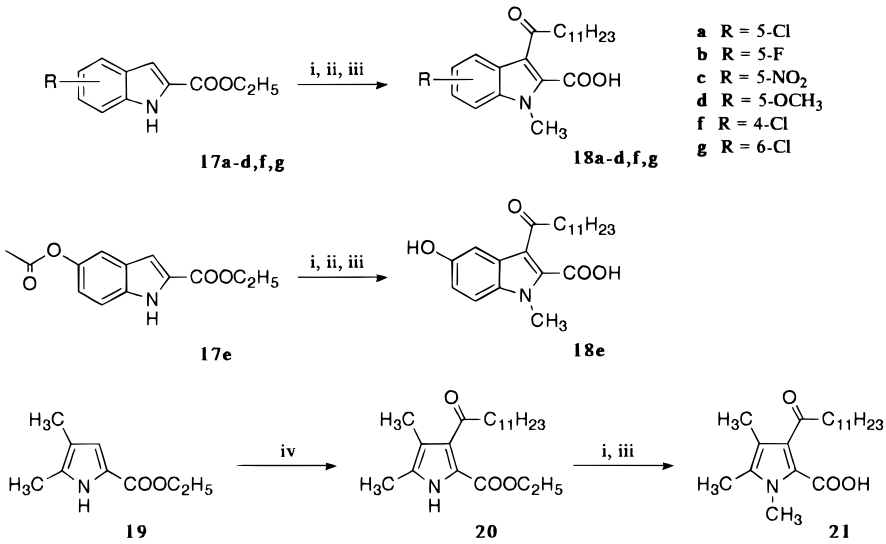
In order to synthesize the pyrrole-2-carboxylic acid **21**, ethyl 4,5-dimethylpyrrole-2-carboxylate (**19**) was acylated with dodecanoyl chloride by the Friedel−Crafts reaction to produce **20** (Scheme 3). This compound was *N*-methylated and hydrolyzed employing reaction conditions similar those described for the synthesis of **9** and **13**.

3-Octadecanoylindole-2-carboxylic acids with different 1-alkyl chains (**23a**−**d**) and 1-(*ω*-carboxyalkyl) chains (**24a**−**g**), respectively, were obtained starting from ethyl indole-2-carboxylate (**22**) (Scheme 4). This was *N*-alkylated with the appropriate 1-bromoalkanes or ethyl *ω*-bromoalkanoates in DMSO in the presence of potassium *tert*-butoxide as base. The ester intermediates were acylated and hydrolyzed in a similar manner as described for **16a**−**m**.

The synthesis of compounds with *m*- and *p*-methyl cinnamic acid or *m*- and *p*-methyl hydrocinnamic acid substituents (**27a,b**, **28a,b**) in position 1 of the indole started from the common intermediates **26a** and **26b**, respectively (Scheme 4). These were hydrolyzed or hydrogenated and hydrolyzed to afford the desired test compounds. The intermediates **26a** and **26b** were prepared on different routes. While **26a** was obtained by treatment of ethyl indole-2-carboxylate (**22**) with ethyl 4-(bromomethyl)cinnamate followed by acylation with dodecanoic acid/trifluoroacetic anhydride/polyphosphoric acid, **26b** was prepared the other way around by first acylating **22** and then coupling the intermediate acyl ester **25** with ethyl 3-(bromomethyl)cinnamate.

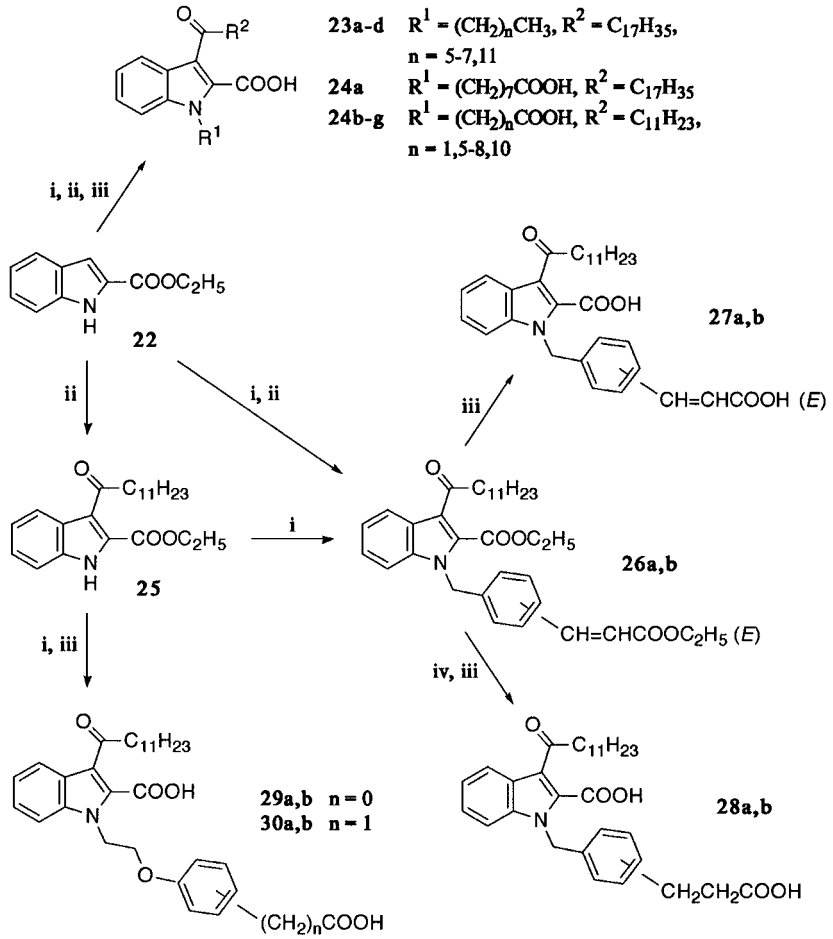
The indoledicarboxylic acid derivatives **29a,b** and **30a,b** were synthesized starting from **25** and the ethyl esters of *m*- or *p*-(2-bromoethoxy)benzoate and *m*- or

Scheme 3



[a] (i) Methyl *p*-toluenesulfonate, $(C_4H_9)_4N^+Br^-$, powdered NaOH, Et_2O, CH_2Cl_2; (ii) dodecanoic acid, trifluoroacetic anhydride, polyphosphoric acid, CH_2Cl_2; (iii) aqueous KOH, EtOH; (iv) dodecanoyl chloride, $AlCl_3$, nitrobenzene.

Scheme 4



[a] (i) 1-Bromoalkane (**23a**−**d**), ethyl *ω*-bromoalkanoate (**24a**−**g**), ethyl 3- or 4-(bromomethyl)cinnamate (**26a,b**), ethyl 3- or 4-(2-bromoethoxy)benzoate (**29a,b**) or ethyl 3- or 4-(2-bromoethoxy)phenylacetate (**30a,b**), *t*-BuOK, DMSO; (ii) octadecanoic or dodecanoic acid, trifluoroacetic anhydride, polyphosphoric acid, CH_2Cl_2; (iii) aqueous KOH, EtOH; (iv) H_2, Pd/C, THF.

p-(2-bromoethoxy)phenylacetate, respectively, applying reaction conditions described above (Scheme 4).

Biological Evaluation

The $cPLA_2$ inhibitory potency 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.[38] In our opinion, such a cellular assay better correlates with the conditions prevailing *in vivo* than a test system using the isolated enzyme. In cells the $cPLA_2$ interacts, once activated, with the substrate in its physiological form

by cleaving phospholipids of the intact cell membranes. On the other hand, in test systems using the isolated enzyme, the $cPLA_2$ hydrolyzes an artificial substrate assembly whose physiological relevance cannot be judged.

The activity of $cPLA_2$ in the cells can be regulated by changes in the intracellular concentration of calcium and by a protein kinase mediated phosphorylation.[26,39] A23187 activates the $cPLA_2$ by causing a rapid increase of the intracellular calcium concentration.[40] Protein kinases are not involved in this mechanism since the protein kinase inhibitor staurosporin did not inhibit A23187-induced arachidonic release (concentration: 1 μM). When using A23187 as stimulant, however, it has to be considered that the decrease of arachidonic acid liberation caused by a test compound may not only be the result of an affection of $cPLA_2$, but can also depend on a modification of the A23187-induced activation mechanism.

To rule out this possibility of action, we measured inhibition of arachidonic acid release by one characteristic compound after stimulation with 12-*O*-tetradecanoylphorbol-13-acetate (TPA).[41] TPA stimulates the $cPLA_2$ in another way than A23187 via phosphorylation of the enzyme as a consequence of an activation of a protein kinase C (PKC) or a mitogen-activated protein kinase (MAP kinase).[42−44]

Recently we have shown that $cPLA_2$ inhibition is simulated when a substance leads to lysis of the platelets.[36] Therefore we also determined the cell lytic potency of each test compound by turbidimetry.

Finally it has to be noted that platelets also contain type II $sPLA_2$.[9] However, this PLA_2 is not involved in the liberation of arachidonic acid.[20−22,41,45] So in combination with the determination of the cell lytic potency and the evaluation of the inhibition of the TPA-induced arachidonic acid liberation, the cellular test system applying A23187 as stimulant is specific for the evaluation of $cPLA_2$ inhibitors.

Structure−Activity Relationships

(A) Variation of the Carboxylic Acid Group. In order to define the structural features necessary for inhibition of $cPLA_2$, we determined the inhibitory potency of the ethyl ester (**4**)[36] of the lead compound **3** (IC_{50}: 8 μM) initially. Since **4** was inactive even at the highest concentration measured (33 μM) (Table 1), it can be assumed that the carboxylic acid group is part of the pharmacophore. Next we replaced the carboxylic acid moiety of **3** and of its desmethyl derivative **5**,[36] respectively, with the homologous acetic acid and propionic acid functions. A significant potency reduction was observed for the 1-methylindoleacetic acid **9** (IC_{50} >33 μM). The 1-methylindolepropionic acid **13** showed no inhibition of $cPLA_2$ at 33 μM. The 1-desmethylindole-2-carboxylic acid **5** and the homologous acetic and propionic acids **8** and **12** also proved to be inactive. The 1-methyl group is therefore essential for the inhibitory activity of the indoles **3** and **9**.

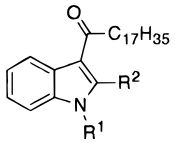
The reference compounds $AACOCF_3$ (**1**) and Wy-48,489 (**2**) inhibited $cPLA_2$ of bovine platelets with an IC_{50} of 11 and 13 μM, respectively. With the exception of the reference substance **1** (31% cell lysis at 33 μM), none of the compounds shown in Table 1 caused cell lysis even up to 33 μM.

(B) Variation of the Acyl Residue. Reduction of the carbonyl of the 3-octadecanoyl chain of **3** to methylene (**14**) led to a total loss of activity (Table 2). This carbonyl function therefore seems to be part of the pharmacophore.

Replacement of the octadecanoyl residue of **3** with a tetradecanoyl and a dodecanoyl chain, respectively, did not change activity: For the compounds **3**, **16a**, and **16b**, an IC_{50} of 8 μM was found in each case. However, a further shortening of the acyl chain led to a decrease of inhibitory potency: While the 3-decanoyl derivative **16c** was about 2-fold less active (IC_{50}: 18 μM) compared to **3**, the IC_{50} of the 3-octanoyl compound **16d** ascended to a value greater than 33 μM (Table 2). Thus the length of the acyl chain in position 3 is of importance for inhibitory activity.

In an attempt to increase potency by conformational restriction, a phenoxy group was incorporated into the acyl chain at various positions. Thus the octadecanoyl moiety of **3** was replaced with benzoyl, phenylacetyl, and 3-phenylpropionyl residues substituted with dodecyloxy

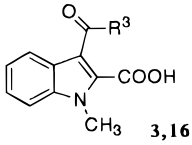
Table 1. *In Vitro* Inhibition of Platelet $cPLA_2$



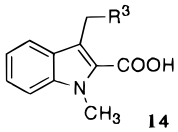
compd	R^1	R^2	cell lysis at 33 μM (%)	IC_{50} (μM)[a]
3	CH_3	COOH	0	8
4	CH_3	$COOC_2H_5$	0	NA[b]
5	H	COOH	0	NA[b]
8	H	CH_2COOH	0	NA[b]
9	CH_3	CH_2COOH	0	>33[c]
12	H	CH_2CH_2COOH	0	NA[b]
13	CH_3	CH_2CH_2COOH	0	NA[b]
$AACOCF_3$ (**1**)			31	11
Wy-48,489 (**2**)			0	13

[a] IC_{50} values are the means of at least two independent determinations. Errors are within ±20%. [b] NA: not active at the highest concentration tested (33 μM). [c] 28% inhibition at 33 μM.

Table 2. *In Vitro* Inhibition of Platelet $cPLA_2$



3,16a-m

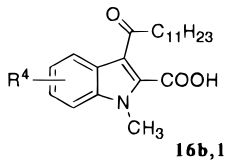


14

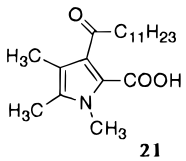
compd	R^3	cell lysis at 33 μM (%)	IC_{50} (μM)[a]
3	$C_{17}H_{35}$	0	8
14	$C_{17}H_{35}$	0	NA[b]
16a	$C_{13}H_{27}$	0	8
16b	$C_{11}H_{23}$	0	8
16c	C_9H_{19}	0	18
16d	C_7H_{15}	0	>33[c]
16e	$C_6H_4(2\text{-}OC_{12}H_{25})$	60	—[d]
16f	$C_6H_4(3\text{-}OC_{12}H_{25})$	26	7
16g	$C_6H_4(4\text{-}OC_{12}H_{25})$	19	7
16h	$CH_2C_6H_4(2\text{-}OC_{10}H_{21})$	37	7
16i	$CH_2C_6H_4(3\text{-}OC_{10}H_{21})$	21	10
16j	$CH_2C_6H_4(4\text{-}OC_{10}H_{21})$	38	7
16k	$CH_2CH_2C_6H_4(2\text{-}OC_{10}H_{21})$	34	8
16l	$CH_2CH_2C_6H_4(3\text{-}OC_{10}H_{21})$	14	11
16m	$CH_2CH_2C_6H_4(4\text{-}OC_{10}H_{21})$	0	10

[a] IC_{50} values are the means of at least two independent determinations. Errors are within ±20%. [b] NA: not active at the highest concentration tested (33 μM). [c] 30% inhibition at 33 μM. [d] IC_{50} could not be determined because of cell lysis even at 10 μM (15%); 26% inhibition of arachidonic acid release at 3.3 μM.

Table 3. *In Vitro* Inhibition of Platelet $cPLA_2$



16b,18a-g



21

compd	R^4	cell lysis at 33 mM (%)	IC_{50} (μM)[a]
16b	H	0	8
18a	5-Cl	14	8
18b	5-F	0	11
18c	5-NO_2	10	10
18d	5-OCH_3	0	8
18e	5-OH	0	10
18f	4-Cl	50	9
18g	6-Cl	26	9
21		0	11

[a] IC_{50} values are the means of at least two independent determinations. Errors are within ±20%.

or decyloxy groups to achieve an overall chain length of about 18 carbon atoms. The alkoxy groups were introduced into the *ortho, meta*, or *para* position of the phenyl substituent, respectively. Surprisingly, all compounds (**16e**−**m**) showed about the same activity as the lead **3**. With one exception (**16m**) the introduction of a phenoxy group led to the appearance of cell lysis at 33 μM. Compound **16e** even destroyed the cells at 10 μM (15% lysis); at the concentration of 3.3 μM, at which lysis could no longer be observed, **16e** inhibited the $cPLA_2$ to about the same extent as the other compounds of this series.

(C) Introduction of Substituents into the Phenyl Ring of the Indole. Next we wanted to study the effect of substituents in the phenyl ring of the 1-methyl-3-octadecanoylindole-2-carboxylic acid (**3**). Since some of the synthesized compounds impaired the determination of the arachidonic acid in the biological assay by overlapping with the arachidonic acid peak in the reversed phase HPLC-chromatogram, we synthesized analogously substituted derivatives of 3-dodecanoyl-1-methylindole-2-carboxylic acid (**16b**). The latter compound was as active as the lead **3**, and the phenyl ring substituted derivatives did not interfere with the arachidonic acid during HPLC analysis.

The following test results were obtained (Table 3): Introduction of a chlorine, fluorine, nitro, methoxy, or hydroxy substituent into position 5 of the indole ring system (**18a**−**e**) did not change activity significantly. Furthermore, chlorine substituents in position 4 or 6 of the indole (**18f,g**) did not alter enzyme inhibition. Therefore, the region of the C5 of the indole does not seem to be essential for activity. This presumption was supported by the fact that the fragment analogue 3-dodecanoyl-1,4,5-trimethylpyrrole-2-carboxylic acid (**21**) was about as active as the indole **16b** and its substituted derivatives, respectively.

The introduction of a chlorine atom in position 4, 5, or 6 and a nitro group in position 5, respectively, led to compounds which caused cell lysis at 33 μM.

(D) Variation of the Substituent in Position 1 of the Indole. As shown in Table 4, replacement of the 1-methyl group of **3** with a dodecyl chain resulted in a total loss of inhibitory activity. The 1-dodecyl derivative **23d** even stimulated calcium ionophore A23187-induced arachidonic acid liberation at 33 μM about 1.25-fold.

On the contrary, substitution of the 1-methyl group of **3** by a hexyl residue (**23a**) retained inhibitory potency. However, lengthening the 1-hexyl residue by one further carbon atom (**23b**) decreased activity (IC_{50} > 10 μM, 34% inhibition at 10 μM), and elongation by two carbons afforded a compound (**23c**) which was inactive at 10 μM.

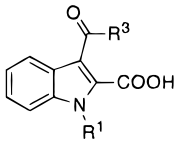
In our opinion, two explanations are possible for these findings: First, the 1-alkyl residue of the indole could be bound in a hydrophobic pocket of the enzyme which can accommodate carbon chains with up to six atoms; introduction of 1-alky substituents with seven or more atoms causes a partial or total loss of activity because of sterical interference. On the other hand, it could be possible that lipophilic 1-alkyl residues with more than six carbons come into close proximity with a hydrophilic part of the enzyme resulting in inactivity because of electronic incompatibility. To obtain evidence for one of these two assumptions, the terminal methyl group of the 1-octyl residue of the indole **23c** should be replaced with a polar moiety. For this exploration we chose the carboxyl function because of the results on structure−activity relationship investigations with 3-(4-acylpyrrol-2-yl)propionic acids: As with the related 3-acylindole-2-carboxylic acids, such pyrroles lost their activity when an alkyl substituent was introduced in position 1 which exceeded a certain chain length (five carbons).[46] Introduction of a polar functional group at the end of the chain, however, restored inhibitory potency in this case. The most preferable of the investigated polar terminal functions (CH_2OH, $CH_2N(CH_3)_2$, $CON(CH_3)_2$, CN, and COOH) was the carboxylic acid moiety. Replacement of the ω-methyl group of the indole **23c** with a carboxylic acid function also gave back activity: With an IC_{50} of 1.4 μM, the dicarboxylic acid **24a** was about 5-fold more active than the lead **3**. These results support the second hypothesis, that substituents in position 1 of the indole-2-carboxylic acids point to a hydrophilic part of the enzyme.

Unfortunately, compound **24a** was highly cytotoxic: At 33 μM it caused 69% cell lysis. However, this cell lytic potency disappeared when the chain length of the 3-acyl residue of **24a** was reduced from 18 to 12 carbons. Since the afforded compound **24e** (IC_{50}: 1.6 μM) was about as active as **24a** (Table 4), it can be assumed that cell lysis, which may be a result of membrane perturbation, and inhibition of calcium ionophore A23187-induced arachidonic acid release are two distinct properties of the compounds.

Next we investigated the consequence of varying the chain length of the octanoic acid substituent of **24e** (Table 4). Elongation of the 1-alkanoic acid up to 11 carbons did not significantly affect activity (**24f,g**), whereas shortening the chain length successively reduced inhibitory potency: The IC_{50} of the 1-heptanoic acid derivative **24d** increased to 3 μM, the compound with a 1-hexanoic acid **24c** inhibited the $cPLA_2$ only to the same extent as 1-methyl derivative **16b** (IC_{50}: 8 μM), and the derivative with an acetic acid function in position 1 was significantly less active (IC_{50} > 33 μM) than **16b**.

Finally the rigidity of the 1-alkanoic acid side chain was increased by introduction of phenyl, ethenylphenyl, and phenoxy groups, respectively (Table 4). The activity of the cinnamic acid and hydrocinnamic acid type derivatives (**27a,b**, **28a,b**) (IC_{50}: 4−5 μM) lay in between the activity of the 1-methyl derivative **16b** (IC_{50}: 8 μM) and of the 1-octanoic acid compound **24e** (IC_{50}:

Table 4. *In Vitro* Inhibition of Platelet $cPLA_2$



compd	R^1	R^3	cell lysis at 33 μM (%)	IC_{50} (μM)[a]
3	CH_3	$C_{17}H_{35}$	0	8
23a	C_6H_{13}	$C_{17}H_{35}$	27	9
23b	C_7H_{15}	$C_{17}H_{35}$	16	>10[b]
23c	C_8H_{17}	$C_{17}H_{35}$	0	NA[c]
23d	$C_{12}H_{25}$	$C_{17}H_{35}$	0	Act[d]
24a	$(CH_2)_7COOH$	$C_{17}H_{35}$	69	1.4
24b	CH_2COOH	$C_{11}H_{23}$	0	>33[e]
24c	$(CH_2)_5COOH$	$C_{11}H_{23}$	0	8
24d	$(CH_2)_6COOH$	$C_{11}H_{23}$	0	3.0
24e	$(CH_2)_7COOH$	$C_{11}H_{23}$	0	1.6
24f	$(CH_2)_8COOH$	$C_{11}H_{23}$	0	1.6
24g	$(CH_2)_{10}COOH$	$C_{11}H_{23}$	0	1.4
27a	$CH_2C_6H_4$(3-CH=CHCOOH)(*E*)	$C_{11}H_{23}$	0	5.3
27b	$CH_2C_6H_4$(4-CH=CHCOOH)(*E*)	$C_{11}H_{23}$	0	4.1
28a	$CH_2C_6H_4$(3-CH_2CH_2COOH)	$C_{11}H_{23}$	0	4.3
28b	$CH_2C_6H_4$(4-CH_2CH_2COOH)	$C_{11}H_{23}$	0	4.0
29a	$CH_2CH_2OC_6H_4$(3-COOH)	$C_{11}H_{23}$	0	1.9
29b	$CH_2CH_2OC_6H_4$(4-COOH)	$C_{11}H_{23}$	0	0.5
30a	$CH_2CH_2OC_6H_4$(3-CH_2COOH)	$C_{11}H_{23}$	0	1.6
30b	$CH_2CH_2OC_6H_4$(4-CH_2COOH)	$C_{11}H_{23}$	0	1.6

[a] IC_{50} values are the means of at least two independent determinations. Errors are within ±20%. [b] 34% inhibition at 10 μM. [c] NA: not active at 10 μM. [d] Act: 1.25-fold activation of arachidonic acid release. [e] 39% inhibition at 33 μM.

1.6 μM). Replacement of the octanoic acid side chain in position 1 with a 2-(3-carboxyphenoxy)ethyl (**29a**), 2-[3-(carboxymethyl)phenoxy]ethyl (**30a**), and 2-[4-(carboxymethyl)phenoxy]ethylresidue (**30b**) maintained inhibitory potency at nearly the same level. However, the introduction of a 2-(4-carboxyphenoxy)ethyl moiety in position 1 of the indole (**29b**) resulted in a 3-fold increase of activity. A reason for this may be that when "locked" to the enzyme the conformational energy of **29b** is significantly lower than that of the other potent compounds with a longer flexible (**24e**−**g**) or semirigid acid substituent in position 1. With an IC_{50} of 0.5 μM compound **29b** was about 20-fold more active than the $cPLA_2$ standard reference inhibitor arachidonyl trifluoromethyl ketone (**1**) (IC_{50}: 11 μM).

To exclude the possibility that the potent indoledicarboxylic acids act by modifying the calcium ionophore A23187-induced activation mechanism of $cPLA_2$ and not by affecting the $cPLA_2$ directly, we measured the inhibition of arachidonic acid release by compound **24e** after stimulation with 12-*O*-tetradecanoylphorbol-13-acetate (TPA) (see Biological Evaluation). Like the lead **3**,[36] compound **24e** reduced the arachidonic acid formation also in this assay. Therefore, it can be safely supposed that the active indoles are actually inhibitors of $cPLA_2$. The IC_{50} for **24e** measured with TPA as stimulant (2.8 μM) was slightly higher than the IC_{50} obtained after calcium ionophore A23187 stimulation of the platelets (1.6 μM). The same effect was observed for the lead **3**[36] and the reference compound **2**.[34]

In the inflamed paws of rats, the concentrations of the arachidonic acid metabolites prostaglandin E_2 and leukotriene B_4 were reduced by compound **24e** to nearly the same extent.[47] These findings indicate that **24e** can inhibit the $cPLA_2$-mediated arachidonic acid release *in vivo* as well.

Model for Inhibitor Binding

Although several inhibitors of $cPLA_2$ have been described in the literature,[32−35] little is known about the way these substances affect enzyme activity. For AACOCF$_3$ (**1**) a direct interaction with the active site of the enzyme was demonstrated by NMR experiments and a crude model for the structure of an enzyme−inhibitor complex was postulated.[32] In this model, the carbon chain of the inhibitor is bound in a hydrophobic pocket and the carbonyl of the AACOCF$_3$ forms a covalent bond with a serine residue of the active site, generating a charged hemiketal oxoanion which interacts with a positively charged group of the enzyme. We have proposed that our lead compound **3** could be bound to the active site in a similar mode:[36] The long carbon chain could lie in the hydrophobic pocket, the carbonyl residue could be bound via a hydrogen bond to the serine residue of the active site, and the carboxylate group could form a salt bridge like the hemiketal oxoanion (Figure 1).

The results of the structure−activity relationship investigation give some support to this model. It can be suggested further on that the carboxylic acid function in position 2 interacts with a positively charged group of the enzyme, since its esterfication leads to a loss of activity. The carbonyl moiety of the 3-octadecanoyl residue also appears to be part of the pharmacophore, because deoxygenation of the acyl residue destroys activity. The fact that a certain length of the acyl chain in position 3 (12 or more carbons) is necessary for optimal activity of the 3-acyl-1-methylindole-2-carboxylic acids provides evidence for the assumption that the acyl residue is bound in a large hydrophobic pocket of the enzyme. The surprising result that constraining the 3-acyl residue in different conformations does not lead to a significant change in activity, however, allows another hypothesis: Possibly there is no specific inter-

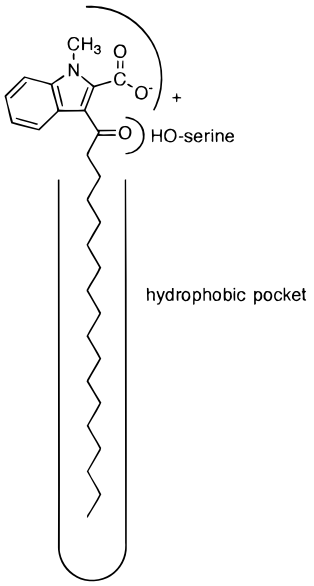


Figure 1. Proposed interaction of 1-methyl-3-octadecanoylindole-2-carboxylic acid (**3**) with the $cPLA_2$ according to the model of Trimble et al.[32]

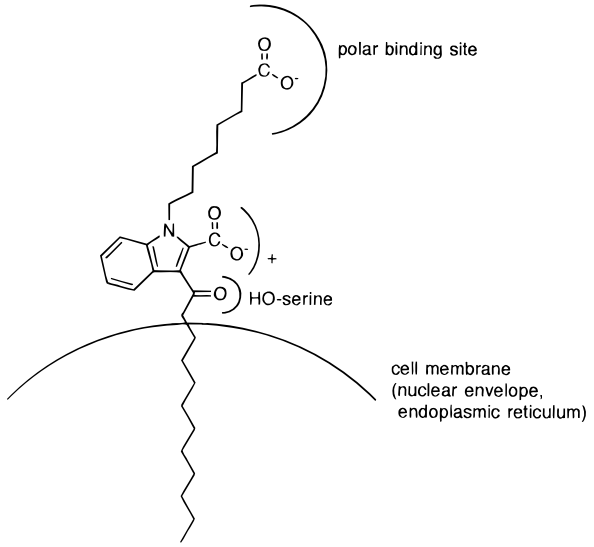


Figure 2. Modified model for the interaction of 3-acylindole-2-carboxylic acids with the $cPLA_2$ using 1-(7-carboxyheptyl)-3-dodecanoylindole-2-carboxylic acid (**24e**) as example.

action between the acyl residue and a hydrophobic pocket of the enzyme. The longer acyl residue is probably only needed for anchoring the indole inhibitor in the cell membrane. A certain chain length of the acyl residue (12 or more carbons) may be necessary to fix the molecules in the plasma membrane optimally, indoles with a shorter acyl chain may be enriched in the lipophilic part of the cell membrane to a lesser extent and therefore the activated enzyme may encounter locally lower concentrations of these compounds resulting in a lower enzyme inhibition. This latter hypothesis correlates with a model of Clark et al.[26] for the catalytic mechanism of the $cPLA_2$. They suggest that during hydrolysis by $cPLA_2$, the lipophilic long acyl or alkyl chain of the phospholipid substrates may remain in the cell membrane (nuclear envelope or endoplasmic reticulum[48]) and that only the region between the scissile ester group and the phosphate group interacts with the enzyme; in this case the selectivity of the $cPLA_2$ for phospholipids with arachidonate in position 2 could be explained with localized packing differences of the different substrate molecules in the cell membrane and would not be due to a specific interaction of the *cis* double bonds of the arachidonate with the active site of the enzyme.

Our results further indicate that the enzyme possesses a polar binding site, which can interact with the ω-carboxylate group of a carboxylic acid substituents in position 1 of the indole insofar as such a residue exceeds a certain length (*e.g.* **24e**).

On the basis of our present data and the published models for inhibitor binding and substrate cleavage,[26,32] for the interaction of the indolecarboxylic acids with the enzyme we now propose the modified model shown in Figure 2.

Further structure−activity relationship investigations will be carried out in order to optimize the activity of the compounds and to evaluate the distance between the binding sites for the two carboxylic acid functions of the indoledicarboxylic acids in particular. For the latter purpose some more derivatives with conformationally constrained carboxylic acid side chain in position 1 of the indole shall be synthesized.

Experimental Section

Chemistry. 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 ionization at 70 eV (EI) or chemical ionization with methane (CI) was applied. Elemental analyses were determined on a Heraeus CHN Rapid instrument and were within ±0.4% of the theroetical value. 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.[33]

Ethyl (3-Octadecanoylindol-2-yl)acetate (7). To the refluxed solution of *N*,*N*-dimethyloctadecanamide (374 mg, 1.2 mmol) and $POCl_3$ (153 mg, 1 mmol) in dry benzene (10 mL) was added in one portion the solution of ethyl (indol-2-yl)-acetate (**6**)[49] (203 mg, 0.4 mmol) in dry benzene (3 mL). After 3 h an aqueous sodium acetate (1 g/4 mL) was added, and refluxing was continued for an additional 15 min with vigorous stirring. The mixture was cooled, diluted with water, and extracted twice with Et_2O. The organic phases were dried and filtered and the filtrate was concentrated. The crude product was purified using silica gel chromatography (elution with petroleum ether−ethyl acetate (1) 9 + 1, (2) 8 + 2) and precipitated from petroleum ether to give **7** as solid: yield 47%; mp 86−87 °C; CI-MS *m/e* 470 $(M + 1)^+$; ^1H-NMR ($CDCl_3$) δ 0.88 (t, 3H), 1.16−1.43 (m, 26H), 1.32 (t, 3H), 1.44 (quint, 2H), 1.78 (quint, 2H), 3.03 (t, 2H), 4.26 (q, 2H), 4.40 (s, 2H), 7.23−7.29 (m, 2H), 7.42−7.44 (m, 1H), 7.88−7.90 (m, 1H), 10.09 (s, 1H).

(3-Octadecanoylindol-2-yl)acetic Acid (8). The mixture of **7** (80 mg, 0.17 mmol), EtOH (15 mL), and 10% aqueous KOH (5 mL) 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 filtered and most of the Et_2O was removed *in vacuo*. After addition of petroleum ether the title compound precipitated: yield 79%; mp 134−136 °C; ^1H-NMR ($CDCl_3$) δ 0.88 (t, 3H), 1.16−1.41

(m, 26H), 1.45 (quint, 2H), 1.83 (quint, 2H), 3.15 (t, 2H), 4.12 (s, 2H), 7.28−7.35 (m, 2H), 7.45−7.47 (m, 1H), 7.81−7.83 (m, 1H), 10.17 (s, 1H). Anal. ($C_{28}H_{43}NO_3$) C, H, N.

(1-Methyl-3-octadecanoylindol-2-yl)acetic Acid (9). The mixture of **7** (80 mg, 0.1 mmol), methyl *p*-toluenesulfonate (32 mg, 0.19 mmol), tetrabutylammonium bromide (22 mg, 0.07 mmol), Et_2O (10 mL), CH_2Cl_2 (5 mL), and powdered NaOH (105 mg, 2.6 mmol) was stirred at room temperature for 20 h. The reaction mixture was filtered and the filter cake washed twice with $Et_2O-CH_2Cl_2$ (1 + 1). The filtrate was evaporated and the residue chromatographed on silica gel with petroleum ether−ethyl acetate (17 + 3) to give the methyl ester of **9**, which was saponified using the same method as for the synthesis of **8**: yield 28%; mp 119−121 °C; ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 3H), 1.09−1.39 (m, 26H), 1.45 (quint, 2H), 1.83 (quint, 2H), 3.15 (t, 2H), 3.91 (s, 3H), 4.09 (s, 2H), 7.33−7.44 (m, 3H), 7.82−7.85 (m, 1H), 13.05 (s, 1H). Anal. ($C_{29}H_{45}NO_3$) C, H, N.

Methyl 3-(3-Octadecanoylindol-2-yl)propionate (11). Preparation started from methyl 3-(indol-2-yl)propionate (**10**)[49] using the same method as for the synthesis of **7**: yield 67%; mp 91−93 °C; CI-MS *m/e* 470 $(M + 1)^+$; ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 3H), 1.16−1.37 (m, 26H), 1.43 (quint, 2H), 1.78 (quint, 2H), 2.83 (t, 2H), 3.02 (t, 2H), 3.46 (t, 2H), 3.67 (s, 3H), 7.21−7.25 (m, 2H), 7.37−7.40 (m, 1H), 7.86−7.88 (m, 1H), 9.22 (s, 1H).

3-(3-Octadecanoylindol-2-yl)propionic Acid (12). Preparation started from **11** using the same method as for the synthesis of **8**: yield 61%; mp 134−136 °C; ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 3H), 1.21−1.37 (m, 26H), 1.44 (quint, 2H), 1.78 (quint, 2H), 2.90 (t, 2H), 3.03 (t, 2H), 3.44 (t, 2H), 7.21−7.28 (m, 2H), 7.37−7.39 (m, 1H), 7.86−7.88 (m, 1H). Anal. ($C_{29}H_{45}NO_3$) C, H, N.

3-(1-Methyl-3-octadecanoylindol-2-yl)propionic Acid (13). Preparation started from **11** in a similar way as described for **9**: yield 47%; mp 106−108 °C; ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 3H), 1.17−1.38 (m, 26H), 1.43 (quint, 2H), 1.79 (quint, 2H), 2.83 (t, 2H), 3.05 (t, 2H), 3.47 (t, 2H), 3.80 (s, 3H), 7.28−7.32 (m, 2H), 7.36−7.40 (m, 1H), 7.86−7.91 (m, 1H). Anal. ($C_{30}H_{47}NO_3$) C, H, N.

1-Methyl-3-octadecylindole-2-carboxylic Acid (14). The mixture of ethyl 1-methyl-3-octadecanoylindole-2-carboxylate (**4**)[36] (94 mg, 0.2 mmol), dry THF (2 mL), dry methyl acetate (3 mL), $NaBH_4$ (40 mg), and $BF_3{\cdot}Et_2O$ (0.2 mL) was stirred at room temperature for 1 h. After addition of 50% MeOH (2 mL), the mixture was stirred for a further 15 min, diluted with water, and extracted twice with Et_2O. The organic phases were dried and filtered, and the filtrate was concentrated to give the ethyl ester of **14**, which was saponified using a similar method as for the synthesis of **8** (deviation: the reaction mixture was refluxed for 1 h, the product was precipitated from MeOH): yield 26%; mp 87−90 °C; ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 3H), 1.11−1.39 (m, 28H), 1.41 (quint, 2H), 1.68 (quint, 2H), 3.15 (t, 2H), 4.05 (s, 3H), 7.13−7.17 (m, 1H), 7.36−7.41 (m, 2H), 7.70 (d, 1H). Anal. ($C_{30}H_{49}NO_2$) C, H, N.

General Procedure for the Synthesis of 3-Acyl-1-methylindole-2-carboxylic Acids (16a−m). The mixture of ethyl 1-methylindole-2-carboxylate[50] (**15**) (122 mg, 0.6 mmol), the appropriate carboxylic acid (0.9 mmol),[51−55] polyphosphoric acid (27 mg), dry CH_2Cl_2 (3 mL), and trifluoroacetic anhydride (0.13 mL) was stirred at room temperature for 4 h. The reaction mixture was diluted with Et_2O, washed with brine and a solution of sodium chloride in 1 M NaOH, dried, and evaporated. The residue was chromatographed on silica gel with petroleum ether−ethyl acetate (**16a,b** 9 + 1; **16c**−**m**, 19 + 1) to give the ethyl ester of **16a**−**m**, which was saponified using a similar method as for the synthesis of **8** (deviation: the reaction mixture was refluxed for 1 h).

1-Methyl-3-tetradecanoylindole-2-carboxylic Acid (16a). The sodium tetradecanoate, which precipitated when the organic phase was washed with the solution of sodium chloride in 1 M NaOH, was filtered off from the organic phase after addition of kieselguhr: yield 14%; mp 118−119 °C; ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 3H), 1.16−1.43 (m, 18H), 1.47 (quint, 2H), 1.86 (quint, 2H), 3.28 (t, 2H), 4.27 (s, 3H), 7.43−7.53 (m, 2H), 7.61 (d, 1H), 8.02 (d, 1H), 16.72 (s, 1H). Anal. ($C_{24}H_{35}NO_3$) C, H, N.

3-Dodecanoyl-1-methylindole-2-carboxylic Acid (16b): yield 23%; mp 116−117 °C. Anal. ($C_{22}H_{31}NO_3$) C, H, N.

3-Decanoyl-1-methylindole-2-carboxylic Acid (16c): yield 23%; mp 114−116 °C. Anal. ($C_{20}H_{27}NO_3$) C, H, N.

1-Methyl-3-octanoylindole-2-carboxylic Acid (16d): yield 38%; mp 113−114 °C. Anal. ($C_{18}H_{23}NO_3$) C, H, N.

3-[2-(Dodecyloxy)benzoyl]-1-methylindole-2-carboxylic Acid (16e): yield 28%; mp 63−65 °C; ^{1}H-NMR ($CDCl_3$) δ 0.72 (quint, 2H), 0.88 (t, 3H), 0.92−1.04 (m, 4H), 1.04−1.15 (m, 2H), 1.15−1.32 (m, 12H), 3.75 (t, 2H), 4.28 (s, 3H), 6.65 (d, 1H), 6.98 (d, 1H), 7.05 (t, 1H), 7.12 (t, 1H), 7.36 (t, 1H), 7.47 (d, 1H), 7.50 (d, 1H), 7.57 (t, 1H), 16.06 (s, 1H). Anal. ($C_{29}H_{37}NO_4$) C, H, N.

3-[3-(Dodecyloxy)benzoyl]-1-methylindole-2-carboxylic Acid (16f): yield 6%; mp 118−120 °C. Anal. ($C_{29}H_{37}NO_4$) C, H, N.

3-[4-(Dodecyloxy)benzoyl]-1-methylindole-2-carboxylic Acid (16g): yield 34%; mp 90−92 °C. Anal. ($C_{29}H_{37}NO_4$) C, H, N.

3-[[2-(Decyloxy)phenyl]acetyl]-1-methylindole-2-carboxylic Acid (16h): yield 40%; mp 77−78 °C; ^{1}H-NMR ($CDCl_3$) δ 0.86 (t, 3H), 0.98−1.32 (m, 14H), 1.58 (quint, 2H), 3.95 (t, 2H), 4.29 (s, 3H), 4.59 (s, 2H), 6.92 (d, 1H), 6.96 (t, 1H), 7.21 (d, 1H), 7.31 (t, 1H), 7.45 (t, 1H), 7.52 (t, 1H), 7.63 (d, 1H), 8.18 (d, 1H). Anal. ($C_{28}H_{35}NO_4$) C, H, N.

3-[[3-(Decyloxy)phenyl]acetyl]-1-methylindole-2-carboxylic Acid (16i): yield 9%; mp 80−81 °C; ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 3H), 1.20−1.38 (m, 12H), 1.44 (quint, 2H), 1.77 (quint, 2H), 3.94 (t, 2H), 4.29 (s, 3H), 4.59 (s, 2H), 6.82−6.85 (m, 3H), 7.26−7.30 (m, 1H), 7.46 (t, 1H), 7.52 (t, 1H), 7.63 (d, 1H), 8.10 (d, 1H). Anal. ($C_{28}H_{35}NO_4$) C, H, N.

3-[[4-(Decyloxy)phenyl]acetyl]-1-methylindole-2-carboxylic Acid (16j): yield 15%; mp 98−99 °C; ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 3H), 1.05−1.32 (m, 12H), 1.45 (quint, 2H), 1.78 (quint, 2H), 3.95 (t, 2H), 4.29 (s, 3H), 4.56 (s, 2H), 6.90 (d, 2H), 7.18 (d, 2H), 7.46 (t, 1H), 7.53 (t, 1H), 7.63 (d, 1H), 8.13 (d, 1H). Anal. ($C_{28}H_{35}NO_4$) C, H, N.

3-[3-[2-(Decyloxy)phenyl]propionyl]-1-methylindole-2-carboxylic Acid (16k): yield 14%; mp 84−85 °C; ^{1}H-NMR ($CDCl_3$) δ 0.86 (t, 3H), 1.08−1.34 (m, 14H), 1.76 (quint, 2H), 3.18 (t, 2H), 3.64 (t, 2H), 3.99 (t, 2H), 4.27 (s, 3H), 6.87 (d, 1H), 6.91 (t, 1H), 7.20−7.26 (m, 2H), 7.39 (t, 1H), 7.48 (t, 1H), 7.59 (t, 1H), 8.02 (d, 1H). Anal. ($C_{29}H_{37}NO_4$) C, H, N.

3-[3-[3-(Decyloxy)phenyl]propionyl]-1-methylindole-2-carboxylic Acid (16l): yield 29%; mp 95−96 °C. Anal. ($C_{29}H_{37}NO_4$) C, H, N.

3-[3-[4-(Decyloxy)phenyl]propionyl]-1-methylindole-2-carboxylic Acid (16m): yield 27%; mp 109−110 °C. Anal. ($C_{29}H_{37}NO_4$) C, H, N.

General Procedure for the Synthesis of 4-, 5-, or 6-Substituted 3-Dodecanoyl-1-methylindole-2-carboxylic Acids (18a−g). The mixture of the appropriate substituted ethyl indole-2-carboxylate (**17a**−**g**) (0.4 mmol),[56−60] methyl *p*-toluenesulfonate (82 mg, 0.44 mmol), tetrabutylammonium bromide (13 mg, 0.04 mmol), Et_2O (5 mL), and powdered NaOH (20 mg, 0.5 mmol) was stirred at room temperature for 6 h. The reaction mixture was filtered, the filter cake washed with $Et_2O-CH_2Cl_2$ (1 + 1), and the filtrate concentrated *in vacuo*. To the residue obtained were added dodecanoic acid (120 mg, 0.6 mmol), polyphosphoric acid (27 mg), dry CH_2Cl_2 (3 mL), and trifluoroacetic anhydride (0.13 mL), and the mixture was stirred at room temperature for 4−24 h. The reaction mixture was diluted with Et_2O, washed with brine and a solution of sodium chloride in 1 M NaOH, dried, and evaporated. The residue was chromatographed on silica gel with petroleum ether−ethyl acetate (**18a**,**b**,**d**,**f**,**g**, 12 + 1, and **18c**,**e**, 9 + 1).The substituted ethyl 3-dodecanoyl-1-methylindolecarboxylate was saponified using a similar method as for the synthesis of **8** (the reaction mixture was refluxed for 15 min to 1 h).

5-Chloro-3-dodecanoyl-1-methylindole-2-carboxylic Acid (18a): yield 29%; mp 105−106 °C; ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 3H), 1.19−1.56 (m, 16H), 1.86 (quint, 2H), 3.22 (t, 2H), 4.25 (s, 3H), 7.47 (dd, 1H), 7.54 (d, 1H), 7.98 (d, 1H). Anal. ($C_{22}H_{30}ClNO_3$) C, H, N.

3-Dodecanoyl-5-fluoro-1-methylindole-2-carboxylic Acid (18b): yield 29%; mp 87−88 °C; ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 3H), 1.05−1.39 (m, 16H), 1.85 (quint, 2H), 3.20 (t, 2H), 4.27 (s, 3H), 7.25−7.30 (m, 1H), 7.57 (dd, 1H), 7.66 (dd, 1H). Anal. ($C_{22}H_{30}FNO_3$) C, H, N.

3-Dodecanoyl-1-methyl-5-nitroindole-2-carboxylic Acid (18c): yield 7%; mp 97−99 °C; ^{1}H-NMR (DMSO-d_6) δ 0.88 (t, 3H), 1.18−1.45 (m, 16H), 1.89 (quint, 2H), 3.34 (t, 2H), 4.31 (s, 3H), 7.71 (d, 1H), 8.39 (d, 1H), 9.03 (s, 1H). Anal. ($C_{22}H_{30}N_2O_5$) C, H, N.

3-Dodecanoyl-5-methoxy-1-methylindole-2-carboxylic Acid (18d): yield 14%; mp 111−113 °C; ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 3H), 1.05−1.29 (m, 14H), 1.48 (quint, 2H), 1.87 (quint, 2H), 3.21 (t, 2H), 3.93 (s, 3H), 4.25 (s, 3H), 7.16 (dd, 1H), 7.35 (d, 1H), 7.51 (d, 1H). Anal. ($C_{23}H_{33}NO_4$) C, H, N.

3-Dodecanoyl-5-hydroxy-1-methylindole-2-carboxylic Acid (18e). The synthesis started from ethyl 5-acetoxyindole-2-carboxylate[57] (**17e**). To dissolve **17e**, CH_2Cl_2 (2.5 mL) was added to the reaction mixture. The *N*-methyl derivative of **17e** was purified by silica gel chromatography (elution with petroleum ether−ethyl acetate, (1) 9 + 1 and (2) 8 + 2). The final product **18e** was precipitated from Et_2O: yield 15%; mp 169−170 °C; ^{1}H-NMR (DMSO-d_6) δ 0.85 (t, 3H), 1.19−1.41 (m, 16H), 1.60 (quint, 2H), 2.81 (t, 2H), 3.80 (s, 3H), 6.85 (dd, 1H), 7.35 (d, 1H), 7.42 (d, 1H), 9.22 (s, 1H). Anal. ($C_{22}H_{31}NO_4$) C, H, N.

4-Chloro-3-dodecanoyl-1-methylindole-2-carboxylic Acid (18f): yield 22%; mp 136−137 °C; ^{1}H-NMR ($CDCl_3$) δ 0.87 (t, 3H), 1.11−1.44 (m, 16H), 1.80 (quint, 2H), 3.02 (t, 2H), 4.08 (s, 3H), 7.20 (t, 1H), 7.30−7.34 (m, 2H). Anal. ($C_{22}H_{30}ClNO_3$) C, H, N.

6-Chloro-3-dodecanoyl-1-methylindole-2-carboxylic Acid (18g): yield 17%; mp 113−114 °C; ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 3H), 1.05−1.37 (m, 16H), 1.85 (quint, 2H), 3.24 (t, 2H), 4.23 (s, 3H), 7.41 (dd, 1H), 7.60 (d, 1H), 7.93 (d, 1H). Anal. ($C_{22}H_{30}ClNO_3$) C, H, N.

Ethyl 3-Dodecanoyl-4,5-dimethylpyrrole-2-carboxylate (20). To a stirred solution of ethyl 4,5-dimethylpyrrole-2-carboxylate (**19**)[61] (1.67 g, 10 mmol) and dodecanoyl chloride (4.38 g, 20 mmol) in dry nitrobenzene was added $AlCl_3$ (2.67 g, 20 mmol). The mixture was allowed to stand for 3 days. Then the mixture was poured into water and extracted twice with CH_2Cl_2. The organic phases were washed with dilute NaOH, dried, and evaporated. Chromatography on silica gel (elution with (1) CH_2Cl_2−petroleum ether, 3 + 1, and (2) petroleum ether−ethyl acetate, 8 + 2) and precipitation from petroleum ether gave **20** as solid: yield 31%; mp 88−90 °C; ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 3H), 1.16−1.37 (m, 16H), 1.33 (t, 3H), 1.65 (quint, 2H), 1.97 (s, 3H), 2.20 (s, 3H), 2.86 (t, 2H), 4.30 (q, 2H), 8.73 (s, 1H).

3-Dodecanoyl-1,4,5-trimethylpyrrole-2-carboxylic Acid (21). The mixture of **20** (175 mg, 0.5 mmol), methyl *p*-toluenesulfonate (102 mg, 0.55 mmol), tetrabutylammonium bromide (16 mg, 0.05 mmol), Et_2O (10 mL), CH_2Cl_2 (6 mL), and powdered NaOH (80 mg, 2 mmol) was stirred at room temperature for 8 h. After addition of water the mixture was extracted twice with Et_2O. The organic phases were dried and evaporated. Chromatography on silica gel (elution with petroleum ether−ethyl acetate, 9 + 1) yielded the ethyl ester of **21** which was saponified using a similar method as for the synthesis of **8** (deviation: the reaction mixture was refluxed for 1 h): yield 58%; mp 83−84 °C; ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 3H), 1.18−1.45 (m, 14H), 1.72 (quint, 2H), 2.23 (s, 3H), 2.29 (s, 3H), 2.91 (t, 2H), 3.95 (s, 3H). Anal. ($C_{20}H_{33}NO_3$) C, H, N.

General Procedure for the Synthesis of 1-Alkyl-3-octadecanoylindole-2-carboxylic Acids (23a−d). The mixture of ethyl indole-2-carboxylate (**22**) (189 mg, 1 mmol), *t*-BuOK (135 mg, 1.2 mmol), and dry DMSO (4 mL) was heated at 110−120 °C for 15 min. After addition of the appropriate 1-bromoalkane (1.2 mmol), the mixture was heated for an additional 15 min at the same temperature. The mixture was cooled, diluted with water, and extracted with Et_2O. The organic phase was dried and the solvent evaporated. To the residue obtained were added octadecanoic acid (427 mg, 1.5 mmol), polyphosphoric acid (67 mg), dry CH_2Cl_2 (6 mL), and trifluoroacetic anhydride (0.33 mL), and the mixture was stirred at room temperature for 4 h. The reaction mixture was diluted with brine and extracted with Et_2O. The extract was washed with a solution of sodium chloride in 1 M NaOH and the precipitated sodium octadecanoate filtered off after addition of kieselguhr. The organic phase was dried and evaporated. The residue was chromatographed on silica gel with petroleum ether−ethyl acetate (**23a−c**, 29 + 1; **23d**, 200 + 1) and the 1-alkylated ethyl 3-octadecanoylindole-2-carboxylate obtained was saponified using a similar method as for the synthesis of **8** (deviation: the reaction mixture was refluxed for 1 h).

1-Hexyl-3-octadecanoylindole-2-carboxylic Acid (23a): yield 13%; mp 75−77 °C; ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 3H), 0.89 (t, 3H), 1.16−1.55 (m, 34H), 1.86 (quint, 4H), 3.27 (t, 2H), 4.79 (t, 2H), 7.44 (t, 1H), 7.49 (t, 1H), 7.59 (d, 1H), 8.01 (d, 1H), 16.67 (br, 1H). Anal. ($C_{33}H_{53}NO_3$) C, H, N.

1-Heptyl-3-octadecanoylindole-2-carboxylic Acid (23b): yield 14%; mp 61−62 °C. Anal. ($C_{34}H_{55}NO_3$) C, H, N.

3-Octadecanoyl-1-octylindole-2-carboxylic Acid (23c): yield 8%; mp 64−65 °C. Anal. ($C_{35}H_{57}NO_3$) C, H, N.

1-Dodecyl-3-octadecanoylindole-2-carboxylic Acid (23d): yield 23%; mp 74−76 °C. Anal. ($C_{39}H_{65}NO_3$) C, H, N.

The 3-acylindole-2-carboxylic acids with an 1-(ω-carboxyalkyl) substituent (**24a−g**) were synthesized similar to **23a−d** using the appropriate ethyl ω-bromoalkanoate[62−64] instead of the 1-bromoalkane. The crude ethyl 1-[ω-(ethoxycarbonyl)-alkyl]indole-2-carboxylate intermediates were purified by chromatography on silica gel (elution with petroleum ether−ethyl acetate, 9 + 1) prior reaction with octadecanoic acid or dodecanoic acid.

1-(7-Carboxyheptyl)-3-octadecanoylindole-2-carboxylic Acid (24a): yield 12%; mp 99−101 °C. Anal. ($C_{35}H_{55}NO_5$) C, H, N.

1-(Carboxymethyl)-3-dodecanoylindole-2-carboxylic Acid (24b): yield 30%; mp 182−183 °C; ^{1}H-NMR ($CDCl_3$) δ 0.86 (t, 3H), 1.11−1.34 (m, 16H), 1.61 (quint, 2H), 2.90 (t, 2H), 5.19 (s, 3H), 7.22 (t, 1H), 7.33 (t, 1H), 7.61 (d, 1H), 7.85 (d, 1H). Anal. ($C_{23}H_{31}NO_5$) C, H, N.

1-(5-Carboxypentyl)-3-dodecanoylindole-2-carboxylic Acid (24c): yield 9%; mp 104−106 °C. Anal. ($C_{27}H_{39}NO_5$) C, H, N.

1-(6-Carboxyhexyl)-3-dodecanoylindole-2-carboxylic Acid (24d): yield 8%; mp 104−105 °C. Anal. ($C_{28}H_{41}NO_5$) C, H, N.

1-(7-Carboxyheptyl)-3-dodecanoylindole-2-carboxylic Acid (24e): yield 12%; mp 109−110 °C; ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 3H), 1.18−1.53 (m, 22H), 1.64 (quint, 2H), 1.82−1.88 (m, 4H), 2.35 (t, 2H), 3.28 (t, 2H), 4.79 (t, 2H), 7.46 (t, 1H), 7.49 (t, 1H), 7.58 (d, 1H), 8.01 (d, 1H). Anal. ($C_{29}H_{43}NO_5$) C, H, N.

1-(8-Carboxyoctyl)-3-dodecanoylindole-2-carboxylic Acid (24f): yield 9%; mp 110−111 °C. Anal. ($C_{30}H_{45}NO_5$) C, H, N.

1-(10-Carboxydecyl)-3-dodecanoylindole-2-carboxylic Acid (24g): yield 15%; mp 103−104 °C. Anal. ($C_{32}H_{49}NO_5$) C, H, N.

Ethyl 3-Dodecanoylindole-2-carboxylate (25). The mixture of ethyl indole-2-carboxylate (**22**) (3.8 g, 20 mmol), dodecanoic acid (6.0 g, 30 mmol), polyphosphoric acid (1.0 mmol), dry CH_2Cl_2 (20 mL) and trifluoroacetic anhydride (4.4 mL) was stirred at room temperature for 4 h. The reaction mixture was diluted with 1 M NaOH and extracted with Et_2O. The organic phase was dried and the solvent evaporated. After addition of petroleum ether **25** precipitated: yield 58%; mp 75−76 °C; EI-MS *m/e* 371 (M^+); ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 3H), 1.13−1.39 (m, 16H), 1.43 (t, 3H), 1.74 (quint, 2H), 3.06 (t, 2H), 4.45 (q, 2H), 7.24 (t, 1H), 7.36 (d, 1H), 7.41 (t, 1H), 7.92 (d, 1H), 9.02 (s, 1H).

Ethyl (*E*)-3-Dodecanoyl-1-[3-[2-(ethoxycarbonyl)ethen-1-yl]benzyl]indole-2-carboxylate (26a). The mixture of **25** (372 mg, 1 mmol), *t*-BuOK (124 mg, 1.1 mmol), and dry DMSO (3 mL) was heated at 110−120 °C for 5 min. After addition of ethyl (*E*)-3-(bromomethyl)cinnamate[65] (296 mg, 1.1 mmol), the mixture was heated for an additional 10 min at the same temperature. The mixture was cooled, diluted with brine, and extracted with Et_2O. The organic phase was dried and the

solvent evaporated. Chromatography on silica gel eluting with petroleum ether−ethyl acetate (9 + 1) gave **26a** as waxlike substance: yield 70%; EI-MS *m/e* 559 (M^+); ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 3H), 1.18−1.48 (m, 22H), 1.77 (quint, 2H), 2.94 (t, 2H), 4.25 (q, 2H), 4.36 (q, 2H), 5.57 (s, 2H), 6.37 (d, 1H), 7.09 (d, 1H), 7.21−7.34 (m, 5H), 7.43 (d, 1H), 7.59 (d, 1H), 7.96 (d, 1H).

Ethyl (*E*)-3-Dodecanoyl-1-[4-[2-(ethoxycarbonyl)ethen-1-yl]benzyl]indole-2-carboxylate (26b). The preparation started from **22** and ethyl (*E*)-4-(bromomethyl)cinnamate[65] using a similar method as for the synthesis of ester intermediates of **24a**−**g**: yield 25%; mp 72−74 °C; EI-MS *m/e* 559 (M^+); ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 3H), 1.17−1.45 (m, 16H), 1.28 (t, 3H), 1.32 (t, 3H), 1.76 (quint, 2H), 2.93 (t, 2H), 4.25 (q, 2H), 4.35 (q, 2H), 5.59 (s, 2H), 6.38 (d, 1H), 7.10 (d, 2H), 7.26−7.35 (m, 3H), 7.44 (d, 2H), 7.62 (d, 1H), 7.95 (d, 1H).

Compounds **27a,b** were synthesized by saponification of **26a,b** using a similar method as for the synthesis of **8** (deviation: the reaction mixture was refluxed for 1 h).

(*E*)-1-[3-(2-Carboxyethen-1-yl)benzyl]-3-dodecanoylindole-2-carboxylic Acid (27a): yield 42%; mp 186−187 °C; ^{1}H-NMR (DMSO-d_6) δ 0.85 (t, 3H), 1.12−1.37 (m, 16H), 1.63 (quint, 2H), 2.91 (t, 2H), 5.63 (s, 2H), 6.45 (d, 1H), 7.10 (d, 1H), 7.22−7.34 (m, 3H), 7.51 (d, 1H), 7.53−7.60 (m, 3H), 7.96 (d, 1H). Anal. ($C_{31}H_{37}NO_5$) C, H, N.

(*E*)-1-[4-(2-Carboxyethen-1-yl)benzyl]-3-dodecanoylindole-2-carboxylic Acid (27b): yield 30%; mp 224−230 °C; ^{1}H-NMR (DMSO-d_6) δ 0.85 (t, 3H), 1.12−1.36 (m, 16H), 1.63 (quint, 2H), 2.91 (t, 2H), 5.65 (s, 2H), 6.46 (d, 1H), 7.15 (d, 2H), 7.24 (t, 1H), 7.29 (t, 1H), 7.50−7.57 (m, 2H), 7.60 (d, 2H), 7.96 (d, 1H). Anal. ($C_{31}H_{37}NO_5$) C, H, N.

1-[3-(2-Carboxyethyl)benzyl]-3-dodecanoylindole-2-carboxylic Acid (28a). A solution of **26a** (56 mg, 0.1 mmol) in THF (5 mL) was treated with a catalytic amount of 10% Pd/C and the mixture hydrogenated at atmospheric pressure for 4 h. After addition of kieselguhr the mixture was filtered and the solvent evaporated. The residue was chromatographed on silica gel with petroleum ether−ethyl acetate (9 + 1), and the intermediate obtained was saponified using a similar method as for the synthesis of **8** (deviation: the reaction mixture was refluxed for 1 h and the product was precipitated from Et_2O): yield 22%; mp 131−132 °C; ^{1}H-NMR (DMSO-d_6) δ 0.85 (t, 3H), 1.14−1.37 (m, 16H), 1.63 (quint, 2H), 2.48 (t, 2H), 2.76 (t, 2H), 2.90 (t, 2H), 5.57 (s, 2H), 6.86 (d, 1H), 7.10 (d, 1H), 7.15−7.31 (m, 4H), 7.55 (d, 1H), 7.96 (d, 1H). Anal. ($C_{31}H_{39}NO_5$) C, H, N.

1-[4-(2-Carboxyethyl)benzyl]-3-dodecanoylindole-2-carboxylic Acid (28b). Preparation analogously **28a** starting from **26b**. The product **28b** was precipitated from petroleum ether: yield 34%; mp 168−169 °C; ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 3H), 1.18−1.42 (m, 14H), 1.48 (quint, 2H), 1.88 (quint, 2H), 2.62 (t, 2H), 2.89 (t, 2H), 3.31 (t, 2H), 6.08 (s, 2H), 7.03 (d, 2H), 7.10 (d, 2H), 7.41−7.46 (m, 2H), 7.54−7.58 (m, 1H), 8.02−8.05 (m, 2H). Anal. ($C_{31}H_{39}NO_5$) C, H, N.

Compounds **29a**,**b** and **30a**,**b** were synthesized in a similar manner as **26a** starting from **25** and applying the appropriate ethyl (2-bromoethoxy)benzoates[66,67] and ethyl [(2-bromoethoxy)phenyl]acetates.[68]

1-[2-(3-Carboxyphenoxy)ethyl]-3-dodecanoylindole-2-carboxylic Acid (29a). After the saponification the product was extracted with $CHCl_3$ and precipitated from Et_2O: yield 43%; mp 195−196 °C; ^{1}H-NMR (DMSO-d_6) δ 0.85 (t, 3H), 1.12−1.34 (m, 16H), 1.61 (quint, 2H), 2.88 (t, 2H), 4.31 (t, 2H), 4.84 (t, 2H), 7.06 (dd, 1H), 7.25 (t, 1H), 7.33−7.39 (m, 3H), 7.48 (d, 1H), 7.76 (d, 1H), 7.92 (d, 1H). Anal. ($C_{30}H_{37}NO_6$) C, H, N.

1-[2-(4-Carboxyphenoxy)ethyl]-3-dodecanoylindole-2-carboxylic Acid (29b). After the saponification the product was extracted with $CHCl_3$ and precipitated from Et_2O: yield 14%; mp 191−192 °C; ^{1}H-NMR (DMSO-d_6) δ 0.85 (t, 3H), 1.13−1.34 (m, 16H), 1.61 (quint, 2H), 2.87 (t, 2H), 4.34 (t, 2H), 4.85 (t, 2H), 6.90 (d, 2H), 7.26 (t, 1H), 7.37 (t, 1H), 7.75 (d, 1H), 7.82 (d, 2H), 7.91 (d, 1H). Anal. ($C_{30}H_{37}NO_6$) C, H, N.

1-[2-[3-(Carboxymethyl)phenoxy]ethyl]-3-dodecanoylindole-2-carboxylic Acid (30a). The product was precipitated from Et_2O-petroleum ether: yield 27%; mp 114−116 °C; ^{1}H-NMR (DMSO-d_6) δ 0.85 (t, 3H), 1.13−1.34 (m, 16H), 1.61 (quint, 2H), 2.88 (t, 2H), 3.47 (s, 2H), 4.23 (t, 2H), 4.80 (t, 2H), 6.71 (d, 1H), 6.72 (s, 1H), 6.79 (d, 1H), 7.16 (t, 1H), 7.25 (t, 1H), 7.36 (t, 1H), 7.74 (d, 1H), 7.93 (d, 1H). Anal. ($C_{31}H_{39}NO_6$) C, H, N.

1-[2-[4-(Carboxymethyl)phenoxy]ethyl]-3-dodecanoylindole-2-carboxylic Acid (30b). The product was precipitated from petroleum ether: yield 20%; mp 130−131 °C; ^{1}H-NMR (DMSO-d_6) δ 0.85 (t, 3H), 1.13−1.37 (m, 16H), 1.62 (quint, 2H), 2.88 (t, 2H), 3.43 (s, 2H), 4.23 (t, 2H), 4.80 (t, 2H), 6.76 (d, 2H), 7.10 (d, 2H), 7.24 (t, 1H), 7.36 (t, 1H), 7.72 (d, 1H, 7.92 (d, 1H). Anal. ($C_{31}H_{39}NO_6$) 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.[38,41] 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 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 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. Compounds **9**, **13,** and **23d** were dissolved in DMSO−0.05 M ethanolic NaOH (1 + 1), all other test compounds were dissolved in DMSO, if necessary with heating. When DMSO−0.05 M NaOH in EtOH was used as solvent, the solution of the test compound (deviating from[38] 10 μL) was added after the platelet suspension to avoid degradation of ETYA. The IC_{50} values (50% inhibitory concentration) were determined graphically and are the means of at least two independent experiments. For $AACOCF_3$ (**1**) the IC_{50} was 11 ± 1.4 μM (mean ± SEM, n = 3), for Wy-48,489 (**2**) it was 13 ± 1.0 μM (mean ± SEM, n = 4), and for **16b** it was 8 ± 0.5 μM (mean ± SEM, n = 5); the inhibition of arachidonic acid release by **16b**, which was included as reference control in each experiment, ranged from 50% to 66% (57 ± 1.3%, mean ± SEM; n = 16) at a concentration of 10 μM. 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.[36] Briefly, to a solution of ETYA was added the test compound solution or the solvent (in 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. Compounds **9**, **13,** and **23d** were dissolved in DMSO−0.05 M ethanolic NaOH (1 + 1); all other test compounds were dissolved in DMSO, if necessary with heating. When DMSO−0.05 M ethanolic NaOH was used as solvent, the solution of the test compound (deviating from[36] 5 μL) was added after the platelet suspension to avoid degradation of ETYA. As reference substance (1-benzyl-3,5-dimethyl-4-octadecanoylpyrrol-2-yl)acetic acid[69] was used. This compound caused a cell lysis of 32 ± 2.7% (mean ± SEM, n = 5, different cell preparations were used). The cell lysis was determined within 36 h after isolation of the platelets. The platelets were stored at +4 °C.

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