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1-(5-Carboxyindol-1-yl)propan-2-one Inhibitors of Human Cytosolic Phospholipase $A_2\alpha$: Effect of Substituents in Position 3 of the Indole Scaffold on Inhibitory Potency, Metabolic Stability, Solubility, and Bioavailability

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Indole-5-carboxylic acids with 3-aryloxy-2-oxopropyl residues in position 1 have been found to be potent inhibitors of human cytosolic phospholipase $A_2\alpha$ ($cPLA_2\alpha$). In the course of structure−activity relationship studies, we investigated the effect of a substitution of indole 3 position with acyl, alkyl, and oxadiazole residues. The highest increase of inhibitory potency could be achieved by a 3-methyl-1,2,4-oxadiazol-5-yl-moiety. Appropriate compound **40** revealed an IC_{50} of 0.0021 μM against isolated $cPLA_2\alpha$. In a cellular assay applying human platelets **40** blocked $cPLA_2\alpha$ activity even with an IC_{50} of 0.0006 μM. Metabolic stability and aqueous solubility of the target compounds were also determined. Furthermore, one selected compound was tested for peroral bioavailability in mice.

Introduction

Cytosolic phospholipase $A_2\alpha$ ($cPLA_2\alpha$[a]) specifically catalyzes the hydrolysis of the *sn*-2 ester of arachidonate-containing membrane phospholipids.[1,2] The released arachidonic acid is rapidly oxidized via cyclooxygenase (COX) and lipoxygenase (LO) pathways to eicosanoids such as prostaglandins and leukotrienes. Remaining lysophospholipids with ether residues in position 1 and choline phosphate head groups in position 3 of the glycerol backbone can be acetylated to platelet activating factor (PAF). Prostaglandins, leukotrienes, and PAF are known to be important mediators of inflammatory processes. Although several other phospholipases A_2 are present in the mammalian organism, the pre-eminence of $cPLA_2\alpha$ for lipid mediator generation was demonstrated especially by studies with $cPLA_2\alpha$ deficient mice. These animals, which display a reduced eicosanoid production, are resistant to disease in a variety of models of inflammation, including collagen-induced arthritis.[3−8] Therefore, $cPLA_2\alpha$ is considered as a target for inflammatory diseases.[9]

Although there have been intense efforts for developing inhibitors of $cPLA_2\alpha$,[10−13] only few substances with high in vitro potency have been found until now, such as certain thiazolidinediones,[14,15] benzhydrylindoles,[16−20] and benzhydrylquinazolinediones[21] as well as 1,3-diaryloxypropan-2-ones like **1** (AR-C70484XX) (Figure 1).[22,23]

We have published effective $cPLA_2\alpha$ inhibitors like compound **2**, structurally related to **1**.[24−28] In these derivatives, heteroaryl substituents are tethered to aryloxypropan-2-one scaffolds. Structure−activity relationship studies have revealed that introduction of a methoxycarbonyl or an acetyl moiety in position 3 of the indole nucleus of compound **2** significantly increases inhibitory potency. IC_{50} of **3** and **4** against $cPLA_2\alpha$ was about 3- and 5-fold lower than that of the lead compound **2**. In this study we investigated the effect of a substitution of the acetyl group of **4** by other acyl and by alkyl residues. Furthermore, we replaced the metabolically less stable ester moiety of **3** by a bioisosteric oxadiazole. Besides inhibition of $cPLA_2\alpha$, metabolic stability and aqueous solubility of the target compounds were determined. Finally, one selected compound was tested for peroral bioavailability in mice.

Chemistry

The preparation of 3-butanoyl-substituted indole **10** started from indole-5-carboxylic acid benzyl ester (**5**) (Scheme 1). After acylation with butanoyl chloride, the obtained intermediate **6** was reacted with epichlorohydrin to afford **7**. Regioselective opening of the epoxy ring of **7** with 4-octylphenol was achieved without solvent in the presence of catalytic amounts of 4-dimethylaminopyridine. Oxidation of the resulting alcohol intermediate **8** to the ketone **9** was carried out with acetic anhydride/DMSO. Hydrogenolytic cleavage of the benzyl ester group of **9** yielded the desired target compound **10**. The same reaction sequence was applied for the synthesis of the 3-hexanoyl-, 3-hexyl-, and 3-(3-carboxypropanoyl)indole-5-carboxylic acid derivatives **11**, **21**, and **22** (Table 1) using the appropriate substituted benzyl indole-5-carboxylates.

Several of the test compounds were prepared starting from the methyl ester of indole-5-carboxylic acid (**24**) as highlighted

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[a] Abbreviations: $cPLA_2\alpha$, cytosolic phospholipase $A_2\alpha$; COX, cyclooxygenase; LO, lipoxygenase; PAF, platelet activating factor; po, peroral; iv, intravenous; THF, tetrahydrofuran; DMSO, dimethylsulfoxide; DMF, dimethylformamide; SAPC, 1-stearoyl-2-arachidonoyl-*sn*-glycero-3-phosphocholine; DOG, 1,2-dioleoyl-*sn*-glycerol; TPA, 12-*O*-tetradecanoylphorbol 13-acetate; ETYA, 5,8,11,14-eicosatetraynoic acid; NDGA, nordihydroguaiaretic acid, UV, ultraviolet; HPLC, high performance liquid chromatography; MS, mass spectrometry; EI, electron beam ionization; ESI, electrospray ionization; log *P*, partition coefficient; NADPH, dihydronicotinamide adenine dinucleotide phosphate; $iPLA_2$, calcium-independent phospholipase A_2; $sPLA_2$, secretory phospholipase A_2.

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for the 5-carboxypentanoyl derivative **30** (Scheme 2). First, position 3 of **24** was substituted with a 5-methoxycarbonylpentanoyl group. Then the 2-oxopropyl substituent was introduced in position 1 of indole **25**, applying the reaction steps described for the synthesis of **9**. Next the ketone group of the oxopropyl residue of intermediate **28** was acetalized with orthoformic acid trimethyl ester in the presence of catalytic amounts of H_2SO_4. The methyl ester groups of obtained compound **29** were saponified with aqueous NaOH. Finally, the acetal protecting group was removed by aqueous HCl in THF to yield the indole-5-carboxylic acid derivative **30**. Similarly, the 3-isobutanoyl, 3-benzoyl-, 3-butyl-, and 3-(4-carboxybutanoyl)-substituted indoles **12**, **14**, **20**, and **23** (Table 1) were obtained. In the case of the 3-(3-carboxybenzoyl)- and 3-(4-carboxybenzoyl) derivatives **31** and **32** (Table 1) this route was slightly modified. Here, the methyl ester protected target compounds were not acetalyzed prior to ester hydrolysis but directly saponified with aqueous KOH in ethanol at room temperature.

For the synthesis of the 3-ethyl-substituted indole **19**, the acetyl group of starting compound **15**[24] was reduced with $NaBH_4/BF_3$ etherate (Scheme 3). The obtained *tert*-butyl 3-ethylindole-5-carboxylate (**16**) was directly reacted with 2-(4-octylphenoxymethyl)oxirane[24] as described recently. Oxidation with acetic anhydride/DMSO and ester cleavage by trifluoroacetic acid gave the desired test compound. Similarly the 3-pivaloyl-substituted indole-5-carboxylic acid **13** (Table 1) was synthesized.

The 3-trifluoroacetyl substituted indole **34** was synthesized from *tert*-butyl-1-[3-(4-octylphenoxy)-2-oxopropyl]indole-5-carboxylate[24] (**33**) in one step by reaction with trifluoroacetic anhydride in CH_2Cl_2 (Scheme 4).

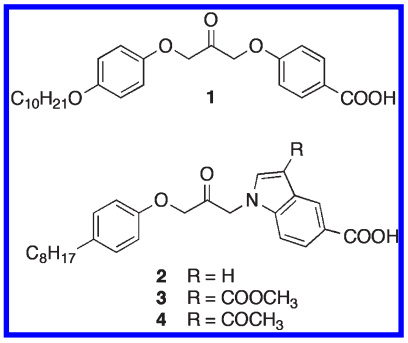


Figure 1

The synthesis of the indole derivative with oxadiazole group in position 3 (**40**) was performed as outlined in Scheme 5. Thus, *tert*-butyl 3-methoxycarbonylindole-5-carboxylate[24] (**35**) was reacted with *N*′-hydroxyacetamidine[29] to afford the oxadiazole-substitued indole **36**. Alkylation of indole 1 position with epichlorohydrin followed by reaction with 4-octylphenol and Dess−Martin oxidation yielded **39**. Finally, the *tert*-butyl ester group was cleaved with trifluororacetic acid to afford target compound **40**.

The 4-phenoxyphenyl-substituted target compounds **41** and **42** (Table 2) were synthesized in a similar way as the corresponding 4-octylphenyl derivatives **30** and **40** (Schemes 2 and 5).

Results and Discussion

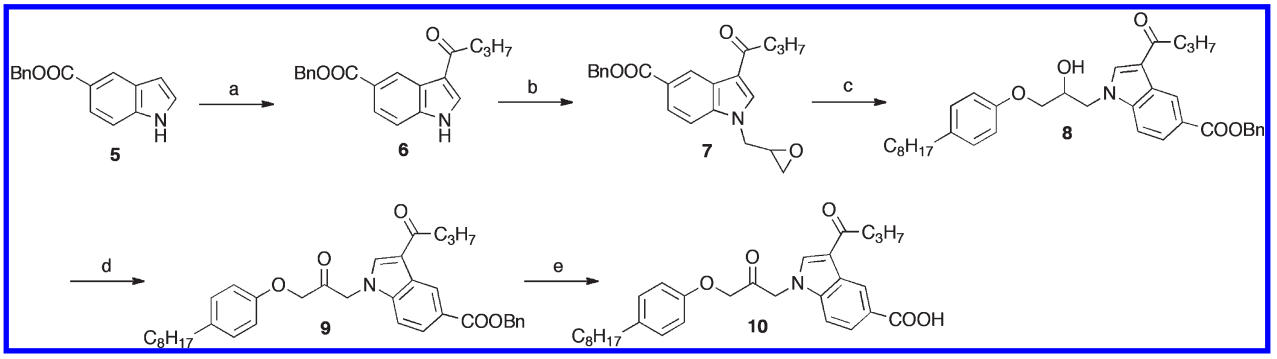
Previously we have shown that introduction of an acetyl group in position 3 of indole-5-carboxylic acid derivative **2** increased cPLA$_2\alpha$ inhibitory potency about 3-fold.[24] Examining the effect of more bulky acyl groups, we found that elongation of the 3-acetyl residue of **4** by two carbons did not change inhibitory potency (Table 1). The 3-butanoyl-substituted compound **10** was as active as **4**. Introduction of one or two methyl substituents in α-position of a propanoyl-residue also did not affect enzyme inhibiton, as shown by the inhibition data of the isobutanoyl- and pivaloyl-substituted derivatives **12** and **13**. In contrast, replacement of the 3-butanoyl residue by the longer 3-hexanoyl moiety (**11**) as well as by the more voluminous benzoyl group (**14**) led to a 5- to 3-fold drop of cPLA$_2\alpha$ inhibition.

In contrast to small acyl residues, small alkyl groups like ethyl and butyl in indole 3 position did not enhance inhibitory activity. With IC_{50} values of about 0.030 μM, **19** and **20** were about as active as the unsubstituted lead compound **2**. Elongation of the butyl residue was not well tolerated by the active site of the enzyme, as shown by the about 20-fold decrease of activity after introduction of a hexyl substituent (**21**).

Replacement of the terminal CH_3 of the highly active 3-butanoyl derivative **10** by a polar carboxy group (**22**) resulted in a similar loss of activity. Interestingly, in the case of the 3-hexanoyl indole-5-carboxylic acid **11**, such a structural modification led to a reversed effect. Here, a carboxy substitutent increased inhibitory potency. This is reflected by the IC_{50} of 0.022 μM of compound **30**.

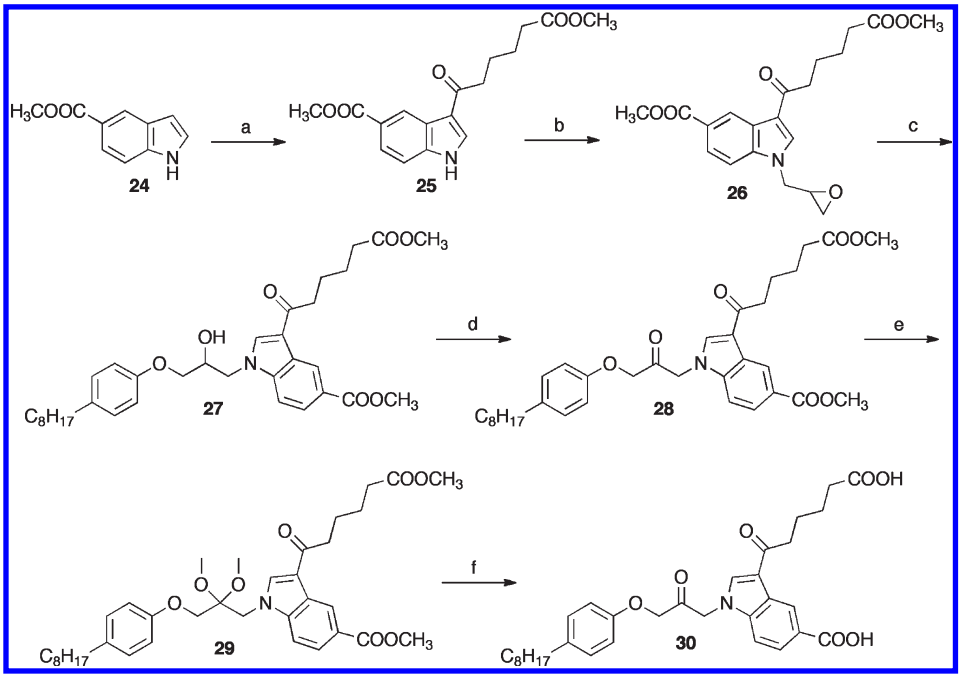
Conformational restriction of the flexible alkyl spacer in the 3-acyl residue of **30** by its replacement by a phenyl residue did not change activity. The comparable activity of the 3-carboxybenzoyl- and the 4-carboxybenzoyl-substituted

Scheme 1[a]



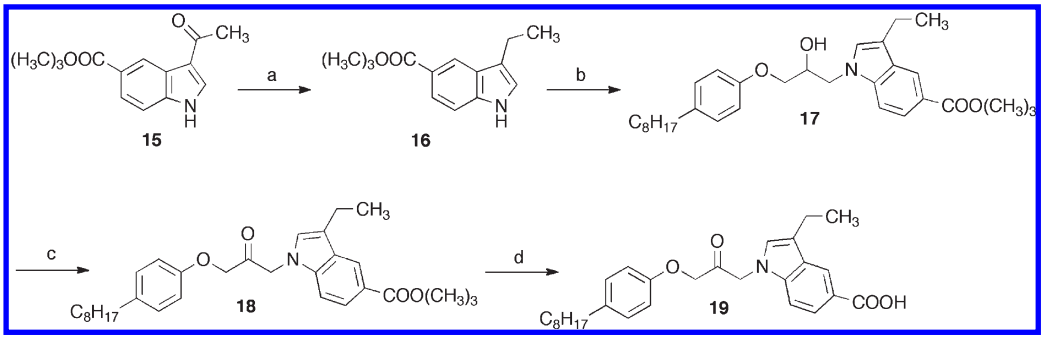
[a] (a) (1) $ZnCl_2$, BuLi, CH_2Cl_2, 0 °C to room temp, (2) butanoyl chloride, $AlCl_3$, 0 °C to room temp; (b) epichlorohydrin, KOH, $Bu_4N^+Br^-$, room temp; (c) 4-octyloxyphenol, 4-dimethylaminopyridine, 100 °C; (d) acetic anhydride, DMSO, room temp; (e) H_2, Pd/C, THF, room temp.

Scheme 2[a]



[a] (a) (1) $ZnCl_2$, BuLi, CH_2Cl_2, 0 °C to room temp, (2) adipic acid monomethyl ester chloride, $AlCl_3$, 0 °C to room temp; (b) epichlorohydrin, KOH, $Bu_4N^+Br^-$, room temp; (c) 4-octylphenol, 4-dimethylaminopyridine, 100 °C; (d) acetic anhydride, DMSO, room temp; (e) trimethyl orthoformate, methanol, H_2SO_4, reflux; (f) (1) aqueous NaOH, methanol, reflux, (2) aqueous HCl, THF, reflux.

Scheme 3[a]



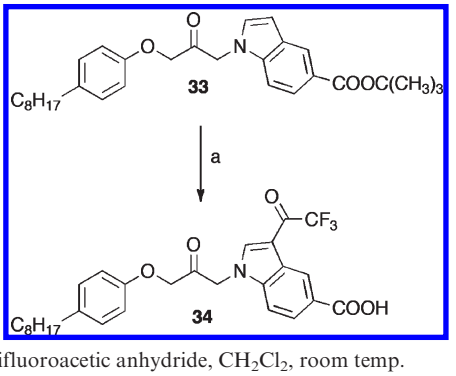
[a] (a) $NaBH_4$, BF_3 etherate, THF; (b) 2-(4-octylphenoxymethyl)oxirane, NaH, DMF, 100 °C; (c) acetic anhydride, DMSO, room temp; (d) trifluoroacetic acid, CH_2Cl_2, room temp.

compounds **31** and **32** indicates that there may be no specific interactions between the carboxylic acid residues of these groups and the enzyme.

Replacement of the 3-acetyl group of **4** by a 3-trifluoroacetyl residue (**34**) increased $cPLA_2\alpha$ inhibition about 2-fold. The exchange of the carboxylic ester group of compound **3** by a bioisosteric 3-methyl-1,2,4-oxadiazol-5-yl moiety alters activity in the same way. With an IC_{50} value of 0.0021 μM, compound **40** is the most potent $cPLA_2\alpha$ inhibitor we have synthesized up to now.

An important structural part of the compounds investigated is their activated electrophilic ketone moiety, which is supposed to form covalent binding interactions with a serine of the active site of $cPLA_2\alpha$.[22,24] Because it is known that activated ketones can be metabolically unstable toward keto reduction[30−32] and because reduction of the ketone groups of our inhibitors to alcohols leads to inactive compounds,[32] we also tested the metabolic stability of all newly synthesized target compounds in a test system applying rat liver microsomes.

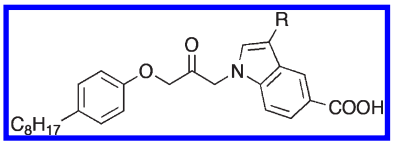
Scheme 4[a]



[a] (a) Trifluoroacetic anhydride, CH_2Cl_2, room temp.

The investigations revealed that introduction of alkyl, acyl, or oxadiazole substituents in indole 3-position of the lead **2** significantly increased stability toward metabolic reduction of the central ketone function.

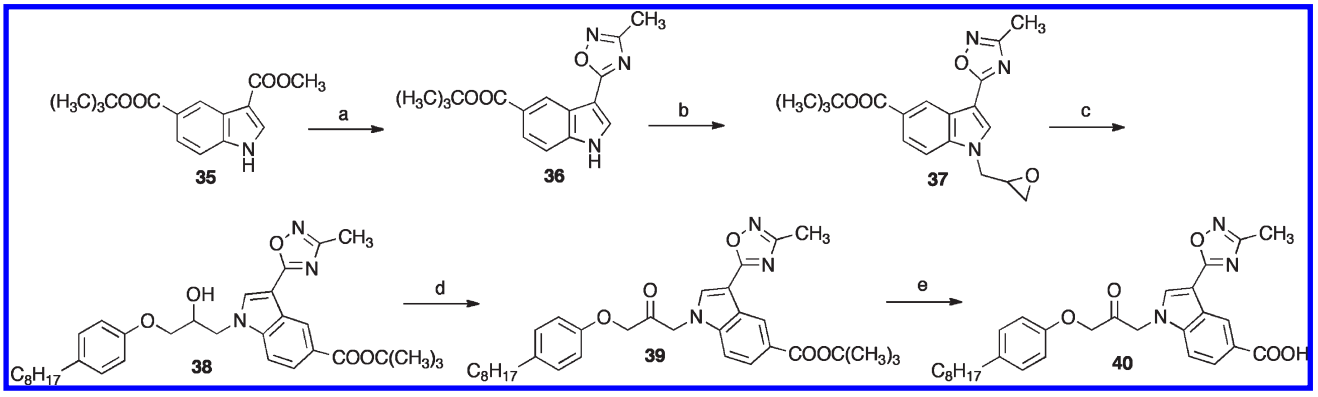
Table 1. cPLA$_2$α Inhibitory Potency, Metabolic Stability, and Aqueous Solubility



compd	R	inhibition of cPLA$_2$α IC$_{50}$ (μM)[a]	metabolic stability (%)[b]	aqueous solubility (μg/mL)[c]	log P[d]
2	H	0.035	65 ± 5	< 1	6.3
3	$COOCH_3$	0.0049	77	< 1	6.2
4	$COCH_3$	0.012	89	< 1	5.8
10	COC_3H_7	0.010	93	< 1	6.7
11	COC_5H_{11}	0.071	93	< 1	> 7
12	$COCH(CH_3)_2$	0.010	82	80 ± 6	6.6
13	$COC(CH_3)_3$	0.013	93	5 ± 2	> 7
14	COphenyl	0.042	95	9 ± 3	6.9
19	C_2H_5	0.030	84	< 1	> 7
20	C_4H_9	0.028	82	< 1	> 7
21	C_6H_{13}	0.59	94	< 1	> 7
22	$CO(CH_2)_2COOH$	0.21	93	354 ± 70	5.1
23	$CO(CH_2)_3COOH$	0.030	95	401 ± 39	5.2
30	$CO(CH_2)_4COOH$	0.022	93	194 ± 34	5.3
31	COphenyl(3-COOH)	0.027	87	12 ± 1	5.7
32	COphenyl(4-COOH)	0.028	87	472 ± 4	5.6
34	$COCF_3$	0.0067	87	< 1	> 7
40	3-methyl-1,2,4-oxadiazol-5-yl	0.0021	81	< 1	6.3

[a] Values are the mean of at least two independent determinations; errors are within ±20%. IC$_{50}$ value of reference inhibitor **1**: 0.011 μM.[22,24] [b] Percentage of parent compound remaining after metabolism by rat liver microsomes. Values are the mean of at least two independent determinations. In the case of **2**: mean ± standard deviation, n = 5. [c] Mean ± standard deviation, n = 3. In the case of values of < 1 μg/mL, n = 2. [d] Partition coefficient (log P) determined by reversed phase HPLC; log P of reference indomethacin, 2.9.

Scheme 5[a]

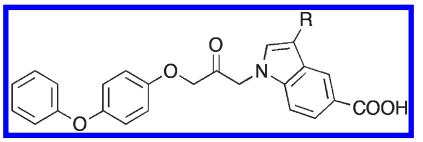


[a] (a) *N*′-Hydroxyacetamidine, NaH, THF, reflux; (b) epichlorohydrin, KOH, $Bu_4N^+Br^-$, room temp; (c) 4-octylphenol, 4-dimethylaminopyridine, 120 °C; (d) acetic anhydride, DMSO, room temp; (e) trifluoroacetic acid, CH_2Cl_2, room temp.

Because of the two long hydrocarbon chains, phospholipid substrates of cPLA$_2$α possess a substantial lipophilicity. Therefore, it can be expected that inhibitors, which will bind competitively to the active site of the enzyme, must possess similar properties. This assumption is confirmed by the fact that most of the known cPLA$_2$α inhibitors with pronounced potency bear larger lipophilic residues,[14−28] which leads to a high total lipophilicity of the compounds. Such a high lipophilicity can cause a low water solubility, which may result in poor drug absorption because the drug does not dissolve sufficiently in the aqueous content of the gastrointestinal tract. Thus, we also measured the aqueous solubility of all new compounds under thermodynamic conditions[33,34] by equilibrating the solids in phosphate buffer (pH 7.4) for 20 h at room temperature, separating nonsoluble material by centrifugation and measuring the soluble aqueous concentration by HPLC. Under these conditions the lead compound **2** showed poor water solubility (< 1 μg/mL) (Table 1). Introduction of linear alkyl or acyl residues in indole 3 position of **2** did not result in compounds with dissolution values higher than 1 μg/mL. However, replacement of the unbranched butanoyl (**10**) by a branched isobutanoyl residue (**12**) significantly increased solubility to a value of 80 μg/mL. Solubility guidelines for drugs under development are given by Lipinski and coworkers.[35] According to those, compounds with mid-range permeability and average potency should possess a minimum thermodynamic solubility of 50 μg/mL. Thus, the isobutanoyl derivative **12** clearly exceeds this limit. The pivaloyl and the benzoyl substituted derivatives **13** and **14** were less soluble but still revealed solubilities higher than 1 μg/mL (5 and 9 μg/mL, respectively).

Introduction of a carboxylic acid group at the end of a propanoyl, butanoyl, or pentanoyl side chain in position 3 of the indole scaffold increased solubility substantially.

Table 2. cPLA$_2$α Inhibitory Potency, Metabolic Stability and Aqueous Solubility



compd	R	inhibition of cPLA$_2$α IC$_{50}$ (μM)[a]	metabolic stability (%)[b]	aqueous solubility (μg/mL)[c]	log P[d]
41	$CO(CH_2)_4COOH$	0.26	24	246 ± 25	2.1
42	3-methyl-1,2,4-oxadiazol-5-yl	0.0065	8	31 ± 2	3.1

[a] Values are the mean of at least two independent determinations; errors are within ±20%. IC$_{50}$ value of reference inhibitor **1**: 0.011 μM.[22,24] [b] Percentage of parent compound remaining after metabolism by rat liver microsomes. Values are the mean of at least two independent determinations. [c] Mean ± standard deviation, n = 3. [d] Partition coefficient (log P) determined by reversed phase HPLC; log P of reference indomethacin, 2.9.

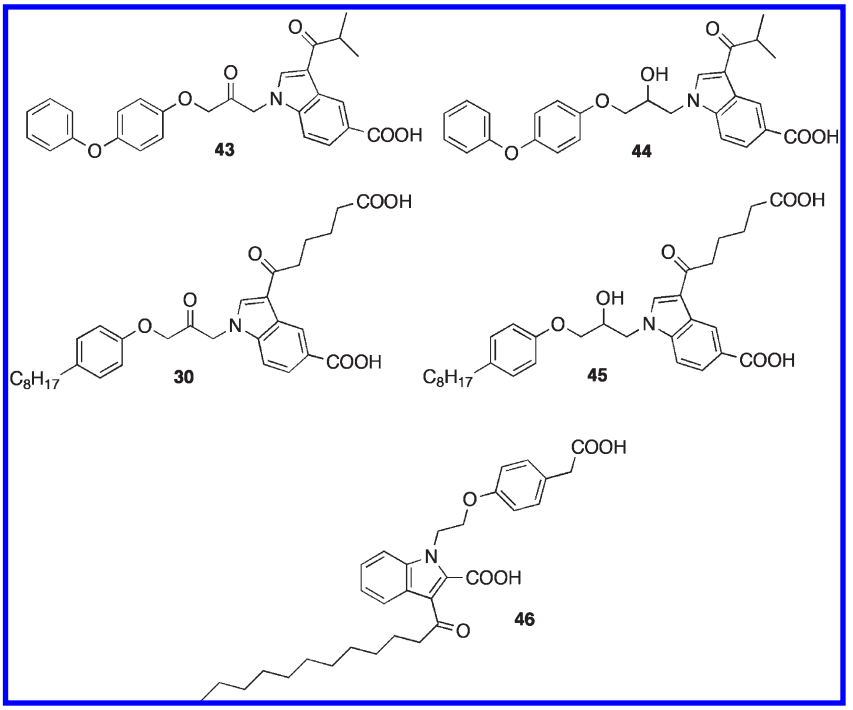


Figure 2

The dissolution values of obtained compounds **22**, **23**, and **30** lay between about 200 and 400 μg/mL. The 4-carboxy substituted benzoyl compound **32** even possessed a value of about 500 μg/mL. Surprisingly, shifting the carboylic acid group of the benzoyl residue of **32** from the para- into the meta-position dramatically decreased aqueous solubility. From the synthesized meta-isomer (**31**) only 12 μg/mL were dissolvable under test conditions.

Recently, we have shown that replacement of the octyl residue of the lead **2** by a phenoxy moiety does not change cPLA$_2$α inhibitory potency but increases aqueous solubility and decreases metabolic stability. We now want to study the effect of such a structural variation on these properties in the cases of the selected compounds **30** and **40**.

As could be expected, the metabolic stability of both phenoxy derivatives (**41** and **42**) was significantly lower than that of the corresponding octyl-substituted parent compounds **30** and **40** (Tables 1 and 2). A substantial increase of water solubility could be determined after replacement of the octyl group of the oxadiazole **40** by a phenoxy residue (**42**). In contrast, in the case of the carboxypentanoyl substituted derivatives **30** and **41**, the amount of dissolvable substance did not change significantly after the same structural variation. Inhibitory potency against cPLA$_2$α dropped in both cases; however, its extent was different. The oxadiazole **42** was about 3-fold less active than its parent compound **40**. In the case of the 5-carboxypentanoyl indoles **30** and **41** the loss of activity was even more than 10-fold. This may be explained by the relatively low log P value (2.1) of **41**. Since cPLA$_2$α in its active status works at the lipophilic phospholipid membrane interface, its inhibitors have to partition into the membrane before they can bind to the enzyme. Because of its low lipophilicity, the ability of **41** to concentrate in the interface could be affected notably.

Compounds **30** and **40** were also tested for cPLA$_2$α inhibition in a cellular situation. In intact human platelets cPLA$_2$α activity triggered by phorbol ester 12-*O*-tetradecanoylphorbol 13-acetate (TPA) was potently inhibited by these target compounds too. The IC$_{50}$ value determined for **30** was 0.076 ± 0.019 μM (mean ± standard deviation, n = 3). Indole **40** inhibited the enzyme even with a subnanomolar IC$_{50}$ of 0.0006 ± 0.0002 μM (mean ± standard deviation, n = 3). For comparison, the potent reference inhibitor **1** showed an IC$_{50}$ of 0.0047 μM in the same assay.[24]

To obtain some information about specifity of **30** for cPLA$_2$α inhibition, we examined its inhibitory potency toward

Table 3. Plasma Levels of the cPLA$_2\alpha$ Inhibitor **30** and Its Alcohol Metabolite **45** after po Administration of 100 mg/kg **30** to Mice and Plasma Levels of the Reference Indomethacin after po Dosage of 10 mg/kg

		plasma level (μg/mL)[a]	
compd	time (h)	**30**	**45**
30 (100 mg/kg)	1	< 0.2	< 0.1
	2	< 0.2	< 0.1
indomethacin (10 mg/kg)	1	42 ± 7.2	
	2	34 ± 5.3	

[a] Values are mean ± standard deviations. Compound **30**: $n = 3$. Indomethacin: $n = 4$.

Table 4. Amount of Parent Compound **30** and Its Alcohol Metabolite **45** Found in Bile and Intestine 2 h after po Administration of **30** (100 mg/kg)

	amount in bile (μg/mL)[a]	
	before treatment with glucuronidase	after treatment with glucuronidase
30	6.0 ± 3.6	33 ± 12
45	< 1	33 ± 13
	amount in intestine (μg/gut)[b]	
	before treatment with glucuronidase	after treatment with glucuronidase
30	870 ± 139	nd[c]
45	14 ± 1.7	nd[c]

[a] Values are the mean ± standard deviations; $n = 3$. [b] Values are the mean ± standard deviations; $n = 3$. The weights of the intestines of the animals were 2.37, 2.22, and 2.12 g, respectively. [c] nd: not determined, since no glucuronides of **30** and **45** could be detected in the intestinal extracts by HPLC.

other PLA$_2$ enzymes, namely, calcium-independent PLA$_2$ (iPLA$_2$) from rat brain cytosol and group IB secretory PLA$_2$ (sPLA$_2$) from porcine pancreas.[36,37] At the highest test concentration of 10 μM, **30** did not inhibit these enzymes, while the applied reference inhibitors for iPLA$_2$ (bromoenol lactone) and sPLA$_2$ ((*S*)-5-(4-benzyloxyphenyl)-4-[(7-phenylheptanoyl)amino]pentanoic acid)[38] showed about 50% and 90% enzyme inhibition, respectively, at this concentration.

Recently, we have investigated the bioavailability of the 5-carboxyindol-1-ylpropan-2-one derivative **43** in mice.[28] Although the compound possessed a considerable aqueous solubility, the median plasma levels of **43** and its main metabolite **44** (Figure 2) determined 1 and 2 h after po administration of 100 mg/kg of **43** were only low (less than 5 μg/mL after 1 h and less than 1 μg/mL after 2 h). For comparison, the concentration of indomethacin in plasma in mice perorally dosed in a concentration of 10 mg/kg was 42 and 34 μg/mL, respectively, after these times.[28] A high plasma clearance of **43** seemed to be the main reason for its impaired po bioavailability. This efficient clearance obviously was due to a pronounced glucuronidation of **43** and **44** in combination with an excessive biliar excretion. One hint for this was the high level of free and bound **43** and **44** found in the bile of the animals. Furthermore, this assumption was supported by the fact that 30 min after iv administration of 10 mg/kg **43** more than 73% of the dose applied could be found in the intestine of the animals in form of **43**, **44**, and their glucuronides.

Because of these results, it could be expected that the bioavailability of other 5-carboxyindol-1-ylpropan-2-ones was also limited. On the other hand, possible exceptions could be the dicarboxylic acid derivatives like **30**, since for structurally related indoledicarboxylic acids, such as compound **46** (Figure 2),[39] high plasma levels (about 100 μg/mL) were observed in rats 4 h after po administration of 100 mg/kg of these substances.[40] Therefore, we also tested the bioavailability of **30**, which possesses a good cPLA$_2\alpha$ inhibitory potency, substantial aqueous solubility, and high metabolic stability against reduction by rat liver microsomes. However, the obtained results were disappointing. The plasma levels measured for **30** and its alcohol metabolite **45** (Figure 2) after 1 and 2 h were even lower than those of **43** and **44** (Table 3).

The total amount of **30**, **45**, and their glucuronides found in bile 2 h after dosage was also significantly lower than that of **43** and its corresponding metabolites. After treatment with glucuronidase, the mean concentration of **30** and **45** in bile together was 66 μg/mL (Table 4) while the biliar concentration levels of **43** and **44** after enzymatic cleavage of the glucuronides were raised to maximal values of 6450 μg/mL.[28]

In the intestine of the mice dosed with **30**, besides only small amounts of the alcohol metabolite **45** (14 μg/gut), high levels of unmetabolized compound **30** could be found (870 μg/gut). Considerable concentrations of glucuronides of these two substances were not detected. In contrast, in the case of **43** the intestinal concentration of the inactive alcohol metabolite **44** in free and glucuronidated form exceeded the concentration of the free and bound parent compound **43** (875 μg/gut vs 404 μg/gut; for details see Supporting Information). The high concentration of unmetabolized **30** in intestine after po administration makes this compound interesting for in vivo testing in animal models of inflammatory bowel diseases. The results of such experiments as well as investigations about topical activity of **30** will be reported in due course.

Experimental Section

1. Chemistry. General. Column chromatography was performed on Merck silica gel 60, 230−400 mesh or 70−230 mesh. Melting points were determined on a Büchi B-540 apparatus and are uncorrected. ^{1}H NMR spectra (400 MHz) and ^{13}C NMR spectra (100 MHz) were recorded on a Varian Mercury Plus 400 spectrometer. Mass spectra were obtained on Finnigan GCQ and LCQ apparatuses applying electron beam ionization (EI) or electrospray ionization (ESI). Purity of the target compounds was determined by normal phase HPLC at 254 nm. For the dicarboxylic acids **22**, **23**, **30**−**32**, and **41** and the trifluoromethyl derivative **34**, a diol phase (LiChrospher 100 DIOL, 5 μm, 3.0 mm (i.d.) × 125 mm, Merck, Darmstadt, Germany) with a heptane/THF gradient at a flow rate of 0.75 mL/min was applied. The other compounds were chromatographed on an amino phase (Spherisorb NH$_2$, 5 μm, 4.0 mm (i.d.) × 250 mm, Latek, Heidelberg, Germany) using an isohexane/THF gradient at a flow rate of 0.75 mL/min. All target compounds showed purities greater or equal 95%, with exception of **10** (purity 92%).

Benzyl 3-Butanoylindole-5-carboxylate (6). To a mixture of a $ZnCl_2$ solution in dry diethyl ether (2.2 M, 2.0 mL) and dry CH_2Cl_2 (10 mL) a solution of butyllithium in dry hexane (1.6 M, 2.4 mL) was slowly added under nitrogen at 0 °C. The mixture was allowed to warm to room temperature, stirred for 1 h at room temperature, treated with a solution of benzyl indole-5-carboxylate[41] (**5**) (1.0 g, 4.0 mmol) in dry CH_2Cl_2 (10 mL), and further stirred for 1 h. The reaction mixture was cooled to 0 °C, butanoyl chloride (0.9 mL, 8.4 mmol) was added, and stirring was continued for 1 h at room temperature. Then the mixture was treated with $AlCl_3$ (0.58 g, 4.4 mmol) and further stirred for 1 h. After addition of half-saturated brine and THF the mixture was extracted exhaustively with ethyl acetate. The combined organic layers were washed with brine, dried (Na_2SO_4), and concentrated. The residue was recrystallized from ethyl acetate to afford **6** as a solid (559 mg, 44%); mp 182−183 °C. ^{1}H NMR (DMSO-d_6) δ 0.92 (t, 3H), 1.65 (sext, 2H), 2.83 (t, 2H), 5.36 (s, 2H), 7.35−7.41 (m, 3H), 7.46−7.49 (m, 2H), 7.55 (d, 1H), 7.85

(dd, 1H), 8.45 (d, 1H), 8.90−8.91 (m, 1H), 12.23 (s_{broad}, 1H). MS (EI): *m*/*z* (%) 321 (84) [M^+], 278 (100).

Benzyl 3-Butanoyl-1-oxiranylmethylindole-5-carboxylate (7). To a mixture of **6** (400 mg, 1.25 mmol), powdered KOH (88%, 140 mg, 2.5 mmol), and tetrabutylammonium bromide (40 mg, 0.13 mmol) was added epichlorohydrin (4 mL). The reaction mixture was stirred at room temperature until **6** had disappeared (about 2 h). The mixture was purified by silica gel chromatography (hexane/ethyl acetate, (a) 9:1, (b) 8:2, (c) 1:1) to give **7** as an oil (411 mg, 87%). ^{1}H NMR ($CDCl_3$): δ 1.00 (t, 3H), 1.79 (sext, 2H), 2.43−2.45 (m, 1H), 2.80−2.85 (m, 3H), 3.30−3.32 (m, 1H), 4.08−4.10 (m, 1H), 4.56 (dd, 1H), 5.39 (s, 2H), 7.31−7.39 (m, 4H), 7.46−7.48 (m, 2H), 7.82 (s, 1H), 8.02 (dd, 1H), 9.13 (d, 1H). MS (EI): *m*/*z* (%) 377 (41) [M^+], 334 (100).

Benzyl 3-Butanoyl-1-[2-hydroxy-3-(4-octylphenoxy)propyl]indole-5-carboxylate (8). Under a nitrogen atmosphere a mixture of **7** (390 mg, 1.03 mmol), 4-octylphenol (213 mg, 1.03 mmol), and 4-dimethylaminopyridine (25 mg, 0.21 mmol) was stirred at 100 °C for 3 h. The reaction mixture was dissolved in toluene and purified by silica gel chromatography (hexane/ethyl acetate, (a) 9:1, (b) 8:2, (c) 1:1) to afford **8** as a solid (192 mg, 32%); mp 74−75 °C. ^{1}H NMR (DMSO-d_6): δ 0.83 (t, 3H), 0.90 (t, 3H), 1.22−1.24 (m, 10H), 1.48−1.49 (m, 2H), 1.63 (sext, 2H), 2.48−2.49 (m, 2H), 2.74 (t, 2H), 3.86−3.89 (m, 2H), 4.19−4.21 (m, 1H), 4.26−4.28 (m, 1H), 4.51−4.53 (m, 1H), 5.36 (s, 2H), 6.84 (d, 2H), 7.07 (d, 2H), 7.39−7.42 (m, 3H), 7.46−7.48 (m, 2H), 7.69 (d, 1H), 7.85 (d, 1H), 8.42 (s, 1H), 8.90−8.91 (m, 1H). MS (EI): *m*/*z* (%) 583 (18) [M^+], 492 (100).

Benzyl 3-Butanoyl-1-[3-(4-octylphenoxy)-2-oxopropyl]indole-5-carboxylate (9). Acetic anhydride (1.1 mL, 12 mmol) was added to dry DMSO (15 mL), and the mixture was stirred under a nitrogen atmosphere at room temperature for 10 min. Then this solution was added drop by drop to a solution of **8** (172 mg, 0.29 mmol) in dry DMSO (15 mL). The mixture was stirred for 18 h under a nitrogen atmosphere and poured into a mixture of 5% aqueous $NaHCO_3$ and half-saturated brine (1:1). After 10 min the mixture was extracted exhaustively with diethyl ether. The combined organic phases were washed three times with brine, dried (Na_2SO_4), and concentrated. The residue was purified by chromatography on silica gel (hexane/ethyl acetate, (a) 9:1, (b) 8:2, (c) 1:1) to give **9** as an oil (79 mg, 48%). ^{1}H NMR (DMSO-d_6): δ 0.84 (t, 3H), 0.93 (t, 3H), 1.22−1.24 (m, 10H), 1.48−1.49 (m, 2H), 1.65 (sext, 2H), 2.48−2.49 (m, 2H), 2.79 (t, 2H), 5.02 (s, 2H), 5.37 (s, 2H), 5.53 (s, 2H), 6.89 (d, 2H), 7.10 (d, 2H), 7.35−7.42 (m, 3H), 7.46−7.49 (m, 2H), 7.59 (d, 1H), 7.86 (dd, 1H), 8.38 (s, 1H), 8.90−8.91 (m, 1H). MS (EI): *m*/*z* (%) 581 (3) [M^+], 377 (100).

3-Butanoyl-1-[3-(4-octylphenoxy)-2-oxopropyl]indole-5-carboxylic Acid (10). A mixture of **9** (75 mg, 0.13 mmol), palladium (10%) on charcoal (19 mg), and THF (10 mL) was stirred under a balloon filled with H_2 at room temperature for 2.5 h. The mixture was filtered through a cotton pad and evaporated. The residue was recrystallized from hexane/ethyl acetate to give **10** as a solid (13 mg, 23%); mp 141−142 °C. ^{1}H NMR (DMSO-d_6): δ 0.83 (t, 3H), 0.92 (t, 3H), 1.21−1.24 (m, 10H), 1.49−1.50 (m, 2H), 1.66 (sext, 2H), 2.49−2.50 (m, 2H), 2.79 (t, 2H), 5.01 (s, 2H), 5.51 (s, 2H), 6.89 (d, 2H), 7.10 (d, 2H), 7.54 (d, 1H), 7.81 (dd, 1H), 8.34 (s, 1H), 8.84−8.85 (m, 1H). ^{13}C NMR (DMSO-d_6): δ 14.51, 14.63, 18.86, 22.76-31.95, 34.90, 41.62, 53.59, 71.55, 111.37, 115.08, 117.26, 124.65, 124.95, 125.30, 125.85, 129.91, 135.79, 139.74, 140.44, 156.38, 168.66, 195.80, 201.27. MS (ESI−): *m*/*z* 490 [M − H]$^-$.

***tert*-Butyl 3-Ethylindole-5-carboxylate (16).** A solution of *tert*-butyl 3-acetylindole-5-carboxylate[24] (**15**) (280 mg, 1.1 mmol) in dry THF (30 mL) was treated with sodium borohydride (81 mg, 2.2 mmol) under a nitrogen atmosphere at 30 °C. Then borontrifluoride etherate (0.4 mL, 3.2 mmol) was added dropwise at such a rate that the temperature did not exceed 45 °C. After being stirred at room temperature for 1 h, the reaction mixture was poured into a mixture of 5% aqueous $NaHCO_3$ and ice−water and extracted exhaustively with diethyl ether. The combined organic phases were dried (Na_2SO_4) and evaporated. Chromatography on silica gel (hexane/ethyl acetate, 8:2) yielded **16** as an oil (236 mg, 89%). ^{1}H NMR ($CDCl_3$): δ 1.33 (t, 3H), 1.63 (s, 9H), 2.80 (q, 2H), 7.01 (s, 1H), 7.32 (d, 1H), 7.85 (dd, 1H), 8.17 (s_{broad}, 1H), 8.33 (s, 1H). MS (EI): *m*/*z* (%) 245 (47) [M^+], 174 (100).

***tert*-Butyl 3-Ethyl-1-[2-hydroxy-3-(4-octylphenoxy)propyl]indole-5-carboxylate (17).** Under a nitrogen atmosphere, a suspension of NaH (60% in mineral oil, 40 mg, 1.01 mmol) in dry DMF (10 mL) was stirred at room temperature for 10 min. After addition of the solution of **16** (236 mg, 0.96 mmol) in dry DMF (10 mL), the mixture was stirred for 1 h. A solution of 2-(4-octylphenoxymethyl)oxirane[24] (252 mg, 0.96 mmol) in dry DMF (10 mL) was added dropwise at room temperature, and the reaction mixture was heated at 60 °C for 4 h. After cooling, the mixture was treated with half-saturated brine and extracted exhaustively with diethyl ether. The combined organic phases were washed with half-saturated brine, dried (Na_2SO_4), and the solvent was evaporated. Chromatography on silica gel with hexane/ethyl acetate (9:1) followed by chromatography on reversed phase silica gel with acetonitrile yielded **17** as an oil (198 mg, 41%). ^{1}H NMR ($CDCl_3$): δ 0.87 (t, 3H), 1.26−1.31 (m, 13H), 1.54−1.61 (m, 11H), 2.51−2.55 (m, 3H), 2.77 (quart, 2H), 3.83 (dd, 1H), 3.90 (dd, 1H), 4.25−4.38 (m, 3H), 6.79 (d, 2H), 6.95 (s, 1H), 7.08 (d, 2H), 7.31 (d, 1H), 7.83 (d, 1H), 8.29 (s, 1H). MS (EI): *m*/*z* (%) 507 (53) [M^+], 451 (100).

***tert*-Butyl 3-Ethyl-1-[3-(4-octylphenoxy)-2-oxopropyl]indole-5-carboxylate (18).** Compound **17** (153 mg, 0.30 mmol) was oxidized according to the procedure described above for the preparation of **9**. The crude product was purified by silica gel chromatography (hexane/ethyl acetate, 9:1) to yield **18** as an oil (77 mg, 66%). ^{1}H NMR ($CDCl_3$): δ 0.89 (t, 3H), 1.24−1.39 (m, 13H), 1.54−1.64 (m, 11H), 2.57 (t, 2H), 2.81 (quart, 2H), 4.59 (s, 2H), 5.13 (s, 2H), 6.80−6.86 (m, 3H), 7.04 (d, 1H), 7.14 (d, 2H), 7.86 (d, 1H), 8.32 (s, 1H). MS (EI): *m*/*z* (%) 505 (2) [M^+], 202 (100).

3-Ethyl-1-[3-(4-octylphenoxy)-2-oxopropyl]indole-5-carboxylic Acid (19). To the solution of **18** (77 mg, 0.15 mmol) in dry CH_2Cl_2 (10 mL) was added trifluoroacetic acid (0.9 mL), and the mixture was stirred at room temperature for 4 h. Then the reaction mixture was concentrated to dryness. The residue was treated twice with hexane, and the solvent was evaporated each time. The residue was recrystallized from hexane/ethyl acetate (6:4) to give **19** as a solid (38 mg, 53%); mp 123−124 °C. ^{1}H NMR (DMSO-d_6): δ 0.83 (t, 3H), 1.22−1.27 (m, 13H), 1.49−1.50 (m, 2H), 2.47−2.49 (m, 2H), 2.72 (q, 2H), 4.96 (s, 2H), 5.34 (s, 2H), 6.86 (d, 2H), 7.09 (d, 2H), 7.13 (s, 1H), 7.36 (d, 1H), 7.69 (dd, 1H), 8.32 (m, 1H), 12.44 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 14.63, 15.08, 18.24, 22.76-31.96, 34.90, 52.85, 71.37, 110.27, 115.02, 118.62, 121.74, 121.80, 123.08, 127.65, 128.21, 129.88, 135.69, 140.00, 156.40, 168.99, 202.10. MS (ESI−): *m*/*z* 448.4 [M − H]$^-$.

Methyl 3-(5-Methoxycarbonylpentanoyl)indole-5-carboxylate (25). A suspension of $AlCl_3$ (4.5 g, 34 mmol) in dry CH_2Cl_2 (40 mL) was treated with adipic acid monomethyl ester chloride (2.5 mL, 15 mmol). After the mixture was stirred at room temperature for 1 h, methyl indole-5-carboxylate (**24**) (1.75 g, 10 mmol) was added and stirring was continued for 30 min. Then water and 1 M HCl were added and the mixture was extracted exhaustively with CH_2Cl_2. The combined organic layers were washed with dilute aqueous Na_2CO_3 solution and water, dried (Na_2SO_4), and concentrated. The residue was recrystallized from ethyl acetate to afford **25** as a solid (2.3 g, 72%); mp 181−182 °C. ^{1}H NMR (DMSO-d_6): δ 1.56−1.66 (m, 4H), 2.34 (t, 2H), 2.86 (t, 2H), 3.56 (s, 3H), 3.85 (s, 3H), 7.53 (d, 1H), 7.81 (dd, 1H), 8.42 (d, 1H), 8.87−8.88 (m, 1H), 12.21 (s_{broad}, 1H). MS (EI): *m*/*z* (%) 317 (13) [M^+], 217 (100).

Methyl 3-(5-Methoxycarbonylpentanoyl)-1-oxiranylmethylindole-5-carboxylate (26). To a mixture of **25** (1.3 g, 4.1 mmol),

powdered KOH (88%, 460 mg, 8.2 mmol), and tetrabutylammonium bromide (132 mg, 0.41 mmol) was added epichlorohydrin (3 mL). The reaction mixture was stirred at room temperature for 1.5 h. The mixture was purified by silica gel chromatography (hexane/ethyl acetate, (a) 9:1, (b) 7:3, (c) 1:1) to give **26** as a solid (939 mg, 61%); mp 140−141 °C. ^{1}H NMR (DMSO-d_6): δ 1.58−1.65 (m, 4H), 2.34 (t, 2H), 2.47−2.49 (m, 1H), 2.79−2.81 (m, 1H), 2.86 (t, 2H), 3.36−3.38 (m, 1H), 3.56 (s, 3H), 3.85 (s, 3H), 4.30 (dd, 1H), 4.66 (dd, 1H), 7.73 (d, 1H), 7.86 (dd, 1H), 8.48 (s, 1H), 8.87−8.88 (m, 1H). MS (EI): m/z (%) 373 (21) [M^+], 273 (100).

Methyl 1-[2-Hydroxy-3-(4-octylphenoxy)propyl]-3-(5-methoxycarbonylpentanoyl)indole-5-carboxylate (27). Under a nitrogen atmosphere a mixture of **26** (900 mg, 2.4 mmol), 4-octylphenol (497 mg, 2.4 mmol), and 4-dimethylaminopyridine (59 mg, 0.48 mmol) was stirred at 100 °C for 30 min. The reaction mixture was dissolved in toluene and purified by silica gel chromatography (hexane/ethyl acetate, (a) 2:1, (b) 1:1) to afford **27** as a solid (0.76 g, 54%); mp 103−104 °C. ^{1}H NMR ($CDCl_3$): δ 0.88 (t, 3H), 1.26−1.30 (m, 10H), 1.55−1.57 (m, 2H), 1.66−1.71 (m, 4H), 2.33 (t, 2H), 2.54 (t, 2H), 2.73 (t, 2H), 3.03 (m, 1H, OH), 3.64 (s, 3H), 3.91 (s, 3H), 3.95−3.99 (m, 2H), 4.31−4.39 (m, 2H), 4.42−4.49 (m, 1H), 6.83 (d, 2H), 7.11 (d, 2H), 7.41 (d, 1H), 7.89 (s, 1H), 7.95 (dd, 1H), 9.01−9.02 (m, 1H). MS (EI): m/z (%) 579 (100) [M^+].

Methyl 3-(5-Methoxycarbonylpentanoyl)-1-[3-(4-octylphenoxy)-2-oxopropyl]indole-5-carboxylate (28). A solution of **27** (0.75 g, 1.3 mmol) in dry CH_2Cl_2 (20 mL) was treated with Dess−Martin periodinane reagent (1.2 g, 2.8 mmol) and stirred under a nitrogen atmosphere at room temperature for 2 h. The reaction mixture was poured into a solution of sodium thiosulfate (3.0 g) in saturated aqueous sodium bicarbonate solution (60 mL) and extracted exhaustively with ethyl acetate. The combined organic layers were dried (Na_2SO_4) and evaporated. The residue was purified by chromatography on silica gel (hexane/ethyl acetate, (a) 2:1, (b) 1.5:1) to yield **28** as a solid (0.69 g, 93%); mp 95−96 °C. ^{1}H NMR ($CDCl_3$): δ 0.88 (t, 3H), 1.27−1.31 (m, 10H), 1.58−1.61 (m, 2H), 1.73−1.81 (m, 4H), 2.38 (t, 2H), 2.58 (t, 2H), 2.88 (t, 2H), 3.66 (s, 3H), 3.93 (s, 3H), 4.71 (s, 2H), 5.30 (s, 2H), 6.87 (d, 2H), 7.09 (d, 1H), 7.17 (d, 2H), 7.75 (s, 1H), 7.97 (d, 1H), 9.10 (s, 1H). MS (EI): m/z (%) 577 (33) [M^+], 373 (100).

Methyl 1-[2,2-Dimethoxy-3-(4-octylphenoxy)propyl]-3-(5-methoxycarbonylpentanoyl)indole-5-carboxylate (29). A solution of **28** (0.69 g, 1.2 mmol) in dry methanol (25 mL) was treated with trimethyl orthoformate (1.5 mL, 13 mmol) and three drops of concentrated H_2SO_4. The mixture was heated under reflux for 3 h. After cooling, the reaction mixture was carefully poured into a 5% aqueous $NaHCO_3$ solution and extracted exhaustively with ethyl acetate. The combined organic phases were dried (Na_2SO_4) and concentrated. The residue was purified by chromatography on silica gel (hexane/ethyl acetate, 8:2) to yield **29** as an oil (183 mg, 24%). ^{1}H NMR ($CDCl_3$): δ 0.87 (t, 3H), 1.25−1.28 (m, 10H), 1.52−1.55 (m, 2H), 1.63−1.72 (m, 4H), 2.32 (t, 2H), 2.50 (t, 2H), 2.64 (t, 2H), 3.41 (s, 6H), 3.66−3.68 (m, 5H), 3.89 (s, 3H), 4.47 (s, 2H), 6.72 (d, 2H), 7.04 (d, 2H), 7.45 (d, 1H), 7.77 (s, 2H), 7.85 (dd, 1H), 9.01−9.02 (m, 1H). MS (ESI+): m/z 624 [$M + H]^+$.

3-(5-Carboxypentanoyl)-1-[3-(4-octylphenoxy)-2-oxopropyl]indole-5-carboxylic Acid (30). To a solution of **29** (180 mg, 0.29 mmol) in methanol (15 mL) was added a solution of NaOH (1.4 g) in water (15 mL). The mixture was heated under reflux for 2.5 h. Then most of the methanol was evaporated in vacuo. The concentrated mixture was acidified with 6 M HCl (17 mL) and extracted exhaustively with ethyl acetate. The combined organic layers were dried (Na_2SO_4) and evaporated. The residue was dissolved in THF (15 mL), treated with 6 M HCl (3 mL), and heated under reflux for 3 h. After the mixture was cooled, water was added (10 mL) and the reaction mixture was extracted exhaustively with ethyl acetate. The combined organic layers were dried (Na_2SO_4) and evaporated. The residue was chromatographed on silica gel (hexane/ethyl acetate/formic acid, (a) 8:2:0.5, (b) 5:5:0.5) to give **30** as a solid (82 mg, 52%); mp 174−175 °C. ^{1}H NMR (DMSO-d_6): δ 0.82 (t, 3H), 1.21−1.24 (m, 10H), 1.48−1.65 (m, 6H), 2.24 (t, 2H), 2.49−2.50 (m, 2H), 2.82 (t, 2H), 5.02 (s, 2H), 5.51 (s, 2H), 6.89 (d, 2H), 7.10 (d, 2H), 7.54 (d, 1H), 7.82 (dd, 1H), 8.36 (s, 1H), 8.84−8.85 (m, 1H), 12.33 (s_{broad}, 2H). ^{13}C NMR (DMSO-d_6): δ 14.64, 22.76-31.94, 34.23, 34.90, 39.32, 53.61, 71.54, 111.40, 115.07, 117.16, 124.62, 124.67, 125.23, 125.86, 129.92, 135.80, 139.77, 140.46, 156.36, 168.64, 175.09, 195.67, 201.24. MS (ESI+): m/z 550 [$M + H]^+$.

1-[3-(4-Octylphenoxy)-2-oxopropyl]-3-(2,2,2-trifluoroacetyl)indole-5-carboxylic Acid (34). To a solution of trifluoroacetic anhydride (9.6 mL) in dry CH_2Cl_2 (140 mL) was added *tert*-butyl 1-[3-(4-octylphenoxy)-2-oxopropyl]indole-5-carboxylate (**33**)[24] (0.69 g, 1.4 mmol) under a nitrogen atmosphere at 0 °C. After being stirred at room temperature for 3 days, the solution was concentrated to half of its volume and triturated with hexane until precipitation occurred. The precipitate was separated by filtration and purified by chromatography on silica gel (hexane/ethyl acetate/formic acid, 3:1:0.1) to give **34** as a solid (0.35 g, 47%); mp 129 °C. ^{1}H NMR ($CDCl_3$): δ 0.88 (t, 3H), 1.27−1.32 (m, 10H), 1.59−1.61 (m, 2H), 2.60 (t, 2H), 4.76 (s, 2H), 5.40 (s, 2H), 6.89 (d, 2H), 7.15−7.20 (m, 3H), 7.96 (s, 1H), 8.11 (dd, 1H), 9.19−9.20 (m, 1H). ^{13}C NMR ($CDCl_3$): δ 14.31, 22.90−32.11, 35.28, 53.99, 72.52, 109.96, 111.54, 114.38, 118.50, 125.37, 126.34, 126.58, 126.96, 130.18, 137.71, 139.00, 140.13, 155.22, 171.92, 175.00, 200.49. MS (ESI+): m/z 518 [$M + H]^+$.

***tert*-Butyl 3-(3-Methyl-1,2,4-oxadiazol-5-yl)indole-5-carboxylate (36).** Under a nitrogen atmosphere, a solution of *N*′-hydroxyacetamidine[29] (135 mg, 1.82 mmol) in dry THF (30 mL) was treated with NaH (60% dispersion in mineral oil) (73 mg, 1.8 mmol) and stirred at room temperature for 1 h. After addition of 5-*tert*-butyl 3-methyl indole-3,5-dicarboxylate (**35**)[24] (500 mg, 1.8 mmol), the mixture was heated under reflux for 24 h. Then water (150 mL) and ethyl acetate (150 mL) were added and most of the remaining THF was evaporated in vacuo. The concentrated mixture was extracted exhaustively with ethyl acetate, and the combined organic layers were dried (Na_2SO_4) and evaporated. The residue was chromatographed on silica gel (hexane/ethyl acetate, 7:3) to afford **36** as a solid (310 mg, 57%); mp 205−206 °C. ^{1}H NMR ($CDCl_3$): δ 1.65 (s, 9H), 2.48 (s, 3H), 7.47 (d, 1H), 7.99 (dd, 1H), 8.10 (m, 1H), 8.97 (m, 1H), 9.20 (s_{broad}, 1H). MS (EI): m/z (%) 299 (36) [M^+], 243 (100).

***tert*-Butyl 3-(3-Methyl-1,2,4-oxadiazol-5-yl)-1-oxiranylmethylindole-5-carboxylate (37).** Compound **36** (305 mg, 1.0 mmol) was reacted with epichlorohydrin as described for the preparation of compound **7**. Chromatography on silica gel (hexane/ethyl acetate, (a) 9:1, (b) 1:1) afforded **37** as a solid (311 mg, 86%); mp 138−139 °C. ^{1}H NMR ($CDCl_3$): δ 1.64 (s, 9H), 2.47−2.50 (m, 4H), 2.86−2.88 (m, 1H), 3.34−3.35 (m, 1H), 4.23 (dd, 1H), 4.57 (dd, 1H), 7.47 (d, 1H), 8.00−8.02 (m, 2H), 8.94 (m, 1H). MS (EI): m/z (%) 355 (48) [M^+], 299 (100).

***tert*-Butyl 1-[2-Hydroxy-3-(4-octylphenoxy)propyl]-3-(3-methyl-1,2,4-oxadiazol-5-yl)indole-5-carboxylate (38).** Under a nitrogen atmosphere a mixture of **37** (150 mg, 0.42 mmol), 4-octylphenol (87 mg, 0.42 mmol), and 4-dimethylaminopyridine (10 mg, 0.08 mmol) was stirred at 120 °C for 1 h. The reaction mixture was dissolved in toluene and purified by silica gel chromatography (hexane/ethyl acetate, 7:3) to afford **38** as an oil (146 mg, 62%). ^{1}H NMR ($CDCl_3$): δ 0.87 (t, 3H), 1.24−1.28 (m, 10H), 1.55−1.58 (m, 2H), 1.64 (s, 9H), 2.44 (s, 3H), 2.54 (t, 2H), 2.78 (s_{broad}, 1H), 3.93−3.99 (m, 2H), 4.38−4.42 (m, 2H), 4.49−4.52 (m, 1H), 6.80 (d, 2H), 7.09 (d, 2H), 7.46 (d, 1H), 7.96 (d, 1H), 8.09 (s, 1H), 8.91 (s, 1H). MS (EI): m/z (%) 561 (1) [M^+], 264 (100).

***tert*-Butyl 3-(3-Methyl-1,2,4-oxadiazol-5-yl)-1-[3-(4-octylphenoxy)-2-oxopropyl]indole-5-carboxylate (39).** Compound **38** (135 mg, 0.24 mmol) was oxidized according to the procedure described above for the preparation of **9**. The crude product was purified

by silica gel chromatography (hexane/ethyl acetate, 8:2) to yield **39** as an oil (48 mg, 38%). ^{1}H NMR ($CDCl_3$): δ 0.88 (t, 3H), 1.27−1.31 (m, 10H), 1.58−1.64 (m, 11H), 2.48 (s, 3H), 2.58 (t, 2H), 4.72 (s, 2H), 5.34 (s, 2H), 6.87 (d, 2H), 7.12 (d, 1H), 7.17 (d, 2H), 7.90 (s, 1H), 7.97 (d, 1H), 8.96 (s, 1H). MS (EI): m/z (%) 559 (1) [M^+], 107 (100).

3-(3-Methyl-1,2,4-oxadiazol-5-yl)-1-[3-(4-octylphenoxy)-2-oxopropyl]indole-5-carboxylic Acid (40). To the solution of **39** (46 mg, 0.08 mmol) in dry CH_2Cl_2 (10 mL) was added trifluoroacetic acid (0.5 mL), and the mixture was stirred at room temperature for 4 h. Then the reaction mixture was concentrated to dryness. The residue was treated twice with hexane, and the solvent was evaporated each time. The residue was recrystallized from ethyl acetate to give **40** as a solid (27 mg, 63%); mp 213−215 °C. ^{1}H NMR (DMSO-d_6): δ 0.83 (t, 3H), 1.22−1.25 (m, 10H), 1.50−1.52 (m, 2H), 2.43 (s, 3H), 2.49−2.50 (m, 2H), 5.03 (s, 2H), 5.60 (s, 2H), 6.91 (d, 2H), 7.11 (d, 2H), 7.66 (d, 1H, ^3J = 8.6 Hz), 7.89 (d, 1H), 8.40 (s, 1H), 8.79 (s, 1H), 12.85 (s_{broad}, 1H). ^{13}C NMR (DMSO-d_6): δ 11.96, 14.63, 22.75−31.93, 34.89, 53.74, 71.49, 101.45, 112.00, 115.07, 123.25, 124.75−124.82, 125.22, 129.92, 135.80, 136.35, 140.26, 156.34, 167.75, 168.42, 172.27, 201.14. MS (ESI+): m/z 504 [M + H]$^+$.

2. Evaluation of the Target Compounds. 2.1. General. The HPLC system applied for measuring enzyme inhibition, metabolic stability, solubility, partition coefficients (log P), and blood plasma concentrations consisted of a Waters HPLC pump model 515, a Waters autosampler model 717 plus, a Waters column oven, and a Waters UV/vis detector model 2487. Instrument control, data collection, and processing were handled by Waters Millenium 32 chromatography software.

2.2. Inhibition of cPLA$_2\alpha$. 2.2.1. Assay with the Isolated Enzyme. Inhibition of cPLA$_2\alpha$ isolated from human platelets was measured as previously published.[42] Briefly, 1-stearoyl-2-arachidonoyl-*sn*-glycero-3-phosphocholine (200 μM) sonicated with 1,2-dioleoyl-*sn*-glycerol (100 μM) in a bath sonicator at 30−35 °C was used as substrate. Enzyme reaction was terminated after 60 min by addition of a mixture of acetonitrile, methanol, and 0.1 M aqueous EDTA−Na_2 solution, which contained 4-undecyloxybenzoic acid as internal standard and nordihydroguaiaretic acid (NDGA) as oxygen scavenger. Released product arachidonic acid was determined with reversed phase HPLC and UV detection at 200 nm after solid phase extraction. Inhibition of cPLA$_2\alpha$ activity was calculated by comparing the arachidonic acid formed by the enzyme in the absence and presence of a test compound.

2.2.2. Cellular Assay. The ability of compounds **30** and **40** to inhibit cPLA$_2\alpha$ activity in intact cells was determined by measuring phorbolester-induced arachidonic acid release from human platelets with HPLC/UV detection according to a method previously described.[33] Briefly, platelets were isolated from human buffy coat by differential centrifugation and washed with phosphate buffered saline (pH 7.4). After addition of the stimulant 12-*O*-tetradecanoylphorbol 13-acetate (TPA) (2 μM) the cells were incubated for 60 min. Incubation was carried out in the presence of a dual cyclooxygenase/12-lipoxygenase inhibitor 5,8,11,14-eicosatetraynoic acid (ETYA) to avoid metabolism of cPLA$_2\alpha$ product arachidonic acid via the cyclooxygenase-1 and the 12-lipoxygenase pathways. Enzyme reaction was terminated by addition of a mixture of acetonitrile, methanol, and 0.1 M aqueous EDTA−Na_2 solution, which contained 3-(4-decyloxyphenyl)propanoic acid as internal standard and nordihydroguaiaretic acid (NDGA) as oxygen scavenger. Inhibition of cPLA$_2\alpha$ activity was determined by comparing the arachidonic acid released by the enzyme in the absence and presence of a test compound with reversed phase HPLC and UV detection at 200 nm after centrifugation of the samples and cleaning up of the supernatant by solid phase extraction.

2.3. Metabolic Stability. Metabolism of the target compounds was studied as described previously using rat liver microsomes.[33] The microsomes were incubated with test substance in the presence and absence of dihydronicotinamide adenine dinucleotide phosphate (NADPH). Incubations were terminated after 30 min by addition of acetonitrile. After vigorous vortexing, the samples were centrifuged and the supernatants were subjected to reversed phase HPLC analysis with UV detection at 240 nm. The metabolic stability was calculated by comparing the amount of the ketone form of the target compound found in the presence and absence of NADPH.

2.4. Solubility. Thermodynamic solubility was determined in the way previously described.[33] Briefly, to 1 mg of a test compound was added phosphate buffered saline (0.1 M, pH 7.4) (2 mL). The mixture was sonicated for 10 min in a bath sonifier and then shaken for 20 h at room temperature. After centrifugation, to an aliquot of the clear supernatant, acetonitrile was added, and the amount of the target compound present in the sample was determined by reversed phase HPLC and UV detection at 240 nm. With this method, for the reference indomethacin an aqueous solubility of 246 ± 18 μg/mL (mean ± standard deviation, n = 4) was measured.

2.5. log *P* Values. Partition coefficients (log P) were determined by reversed phase HPLC according to an OECD guideline,[43] applying the specific procedure published recently.[28] With this method, for indomethacin and ibuprofen log P values of 2.9 and 3.2, respectively, were obtained. For determination of the log P values of the target compounds, the retention times of the peak of their ketone forms were used.

2.6. Inhibition of Calcium-Independent Phospholipase A$_2$ (iPLA$_2$) and Secretory Phospholipase A$_2$ from Porcine Pancreas (sPLA$_2$ Group IB). Inhibition of iPLA$_2$ in rat brain cytosol and sPLA$_2$ from porcine pancreas (group IB) by **30** was assessed by the methods described recently.[28,36,37] Briefly, in the case of iPLA$_2$, 1-palmitoyl-2-(10-pyren-1-yldecanoyl)-*sn*-glycero-3-phosphocholine was used as substrate. Enzyme activity in the presence and absence of the test compound was determined by measuring the release of 10-pyren-1-yldecanoic acid by reversed phase HPLC and fluorescence detection. Inhibition of sPLA$_2$ IB was evaluated with the substrate 2-oleoyl-1-palmitoyl-*sn*-glycero-3-phospho-*rac*-(1-glycerol) (ammonium salt) in the presence of sodium deoxycholate. The release of the enzyme product oleic acid was monitored by reversed phase HPLC and UV detection at 200 nm. Bromoenol lactone (Cayman, no. 70700) and (*S*)-5-(4-benzyloxyphenyl)-4-[(7-phenylheptanoyl)amino]pentanoic acid (Sigma-Aldrich, no. S3319), respectively, were tested as reference inhibitors.

2.7. Bioavailability Studies. 2.7.1. Determination of 30 and Its Metabolite 45 in Mouse Plasma. The pharmacokinetic studies with compound **30** were performed with C57Bl6 mice. Animals (n = 3) were dosed perorally once via gavage with 100 mg/kg **30** (8 μL per gram of mouse of a suspension of 12.5 mg of **30** in 1000 μL of a 1% aqueous methylcellulose solution, Methocel A4C, 408 mPas, Fa. Colorcon). Blood samples were withdrawn from each animal after 1 and 2 h. All blood samples were centrifuged at 3000g for 20 min, and the obtained plasma (about 100 μL each) was separated.

To 50 μL of each blood plasma were added 200 μL of phosphate buffered saline (pH 7.4) and 250 μL of acetonitrile. After vortexing, the mixtures were allowed to stand for 5 min at room temperature. Then the samples were centrifuged at 2000g for 15 min, and the supernatants were subjected to HPLC analysis. Separation was achieved on a Phenomenex Aqua C18 analytical column (4.6 mm inside diameter × 100 mm, particle size 3 μm) protected with a Phenomenex C18 security guard column (3 mm inside diameter × 4 mm). A 100 μL aliquot of each sample was injected into the HPLC system. The mobile phase consisted of acetonitrile/water/phosphoric acid (85%) (70:30:0.1, v/v/v). The flow rate was 0.7 mL/min, and absorption wavelength was set to 235 nm. The amounts of **30** and its alcohol metabolite **45** were determined using standard curves of the compounds.

Control experiments with indomethacin had been performed before in the same way dosing the mice ($n = 4$) perorally with 10 mg/kg (8 μL per gram of mouse of a suspension of 2.5 mg of indomethacin in 2000 μL of a 1% aqueous methylcellulose solution).[28]

2.7.2. Determination of 30 and 45 and Their Glucuronides in the Bile of the Mice. In the pharmacokinetic studies with **30**, the animals were killed after the final blood withdrawal (2 h after po administration), and bile of each animal was collected.

2.7.2.1. Direct Measurement of 30 and 45 in Bile. To 2 μL of the bile samples were added 98 μL of phosphate buffered saline (pH 7.4) and 100 μL of acetonitrile. After being vortexed, the mixtures were allowed to stand for 5 min at room temperature. Then the samples were centrifuged at 2000*g* for 15 min and the supernatants were subjected to HPLC analysis as described above (see section 2.7.1.). The injection volume was 100 μL.

2.7.2.2. Measurement of 30 and 45 in Bile after Treatment with Glucuronidase. To 2 μL of the bile samples were added 178 μL of phosphate buffered saline (pH 5.5) and 20 μL of a solution of β-glucuronidase type HP-2 from *Helix pomatia* (aqueous solution, ≥100000 units/mL, Sigma-Aldrich, no. G7017). The mixtures were heated in a water bath at 60 °C for 1 h. After addition of 200 μL of acetonitrile, the samples were allowed to stand for 5 min at room temperature. Then they were centrifuged at 2000*g* for 15 min and the supernatants were subjected to HPLC analysis as described above (see section 2.7.1.). The injection volume was 200 μL.

2.7.2.3. Measurement of the Glucuronides of 30 and 45 in Bile with HPLC/MS. The obtained solutions were also investigated by HPLC/high resolution mass spectrometry according to the procedure described previously.[28] The samples, which had not been treated with glucuronidase, contained glucuronides of **30** and **45**, as confirmed by their high resolution mass peaks of m/z 724.3004 and m/z 726.3146 ($[M - H]^-$), respectively. In the samples treated with glucuronidase increased concentrations of **30** (m/z 548.2685, $[M - H]^-$) and **45** (m/z 550.2833, $[M - H]^+$) occurred, while their glucuronides could not be detected anymore.

2.7.3. Determination of 30 and 45 in the Intestine of the Mice. After killing of the mice (2 h after po administration of **30**), the whole intestines of the animals were withdrawn. The weights of the intestines were 2.37, 2.22, and 2.12 g. Each intestine was crushed with a scalpel and transferred into a centrifuge tube with phosphate buffered saline (pH 7.4) (2.5 mL). After homogenization of the sample with an Ultra Turrax mixer at 0 °C, acetonitrile (3 mL) was added. The homogenate was vortexed and centrifuged at 2000*g* and 4 °C for 5 min. The supernatant was transferred into a 20 mL volumetric flask. The Ultra Turrax mixer was washed with phosphate buffered saline (pH 7.4)/acetonitrile, 1:1 (v/v) (4 mL). The washing liquid was given to the centrifugation pellet. After being vortexed, the mixture was centrifuged at 2000*g* and 4 °C for 5 min and the supernatant was also transferred into a 20 mL volumetric flask. This washing process was repeated twice. Then the volumetric flask was filled up with phosphate buffered saline (pH 7.4)/acetonitrile, 1:1 (v/v). An aliquot of the extract was centrifuged at 2000*g* and room temperature for 5 min, filtered through a little cotton pad, and subjected to HPLC analysis as described above (see section 2.7.1). The injection volume was 20 μL.

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Supporting Information Available: Synthesis and analytical data of the target compounds **11**−**14**, **20**−**23**, **31**, **32**, **41**, **42**, and their intermediates; synthesis and analytical data of the alcohol metabolite **45**; HPLC−UV chromatograms of bile and intestine samples of mice perorally dosed with 100 mg/kg **30**; method for determination of **43** and **44** in the intestine of mice treated with **43**; HPLC−UV chromatograms of intestinal samples of a mouse perorally dosed with 100 mg/kg of **43**. This material is available free of charge via the Internet at http://pubs.acs.org.

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