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1-(5-Carboxyindol-1-yl)propan-2-one Inhibitors of Human Cytosolic Phospholipase $A_2\alpha$ with Reduced Lipophilicity: Synthesis, Biological Activity, Metabolic Stability, Solubility, Bioavailability, And Topical in Vivo Activity

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Indole-5-carboxylic acids with 3-aryloxy-2-oxopropyl residues in position 1 were previously reported to be potent inhibitors of human cytosolic phospholipase $A_2\alpha$ ($cPLA_2\alpha$). In continuation of our attempts to develop clinical active $cPLA_2\alpha$ inhibitors, a series of structurally related indole-5-carboxylic acids with reduced lipophilicity was synthesized and tested for $cPLA_2\alpha$-inhibitory potency. Furthermore, the thermodynamic solubility of these compounds and their metabolic stability in rat liver microsomes were evaluated. With an IC_{50} of 0.012 μM against the isolated enzyme, compound **36** was one of the most potent $cPLA_2\alpha$ inhibitors that emerged during the structure−activity relationship study. Concomitantly, **36** possessed the highest water solubility (212 μg/mL at pH 7.4) of all new target compounds. Despite these favorable properties, peroral application of **36** (100 mg/kg) in mice only led to low concentrations of the substance in blood plasma. A very high plasma clearance was observed after intravenous administration of **36** (10 mg/kg). However, in a topical murine model of contact dermatitis, **36** showed a pronounced anti-inflammatory in vivo activity.

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 a few substances with high in vitro potency have been found until now, such as the thiazolidinedione **1** of Shionogi,[14,15] the benzhydrylindole **2** (efipladib) of Wyeth,[16−19] and the 1,3-diaryloxypropan-2-one **3** (AR-C70484XX) of AstraZeneca (Figure 1).[20]

Meanwhile, also some in vivo data of $cPLA_2\alpha$ inhibitors have been published. Thiazolidinedione **1** was found to be active in the 2,4,6-trinitro-1-chlorobenzene (TNBC)-induced ear inflammation model in mice.[21] A structurally related substance displayed antiarthritic and antibone destructive action in a murine arthritis model when administered perorally.[22] Furthermore, several indole derivatives of Wyeth like compound **2** also showed peroral (po) activity in several in vivo inflammation models, namely the rat carrageenan-induced paw edema model, the rat carrageenan air pouch model, and the murine collagen-induced arthritis model.[17−19] Moreover, certain 2-oxoamide-based compounds were reported to have therapeutic effects in animal models of pain and inflammation after intraperitoneal (ip) or intrathecal (i.t.) administration.[23,24]

A common drawback of these inhibitors is their high lipophilicity, which leads to low aqueous solubility and as a consequence of this to poor po bioavailability. To achieve a sufficient blood concentration of these substances after po administration, lipid-, detergent-, or solvent-based formulations

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[a]Abbreviations: $cPLA_2\alpha$, cytosolic phospholipase $A_2\alpha$; COX, cyclooxygenase; LO, lipoxygenase; PAF, platelet activating factor; po, peroral; ip, intraperitoneal; i.t., intrathecal; iv, intravenous; THF, tetrahydrofuran; DMSO, dimethyl sulfoxide; 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; BAC, benzalkonium chloride; NADPH, dihydronicotinamide adenine dinucleotide phosphate; $iPLA_2$, calcium-independent phospholipase A_2; $sPLA_2$, secretory phospholipase A_2.

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have to be used.[17−19,22] For this reason, efforts have been started to lower the lipophilicity of such cPLA$_2\alpha$ inhibitors.[25−27] However, lowering of lipophilicity often is accompanied by a reduction of enzyme inhibitory potency. This is consistent with the fact that cPLA$_2\alpha$ in its active status works at the lipophilic phospholipid membrane interface and its inhibitors have to partition into the membrane before they can bind to the enzyme. For enrichment in the phospholipid bilayer, however, the inhibitors must possess a substantial lipophilicity.

Recently, we have published effective cPLA$_2\alpha$ inhibitors like compounds **4**−**6**, structurally related to **3**.[28] In these derivatives, 5-carboxyindol-1-yl or 5-carbamoylindol-1-yl substituents are tethered to aryloxypropan-2-one scaffolds. Like other potent inhibitors of cPLA$_2\alpha$, compounds **4**−**6** possess a very high lipophilicity. Main lipophilic elements of **4**−**6** are their octyl and decyloxy substituents, respectively. In this study, we tried to obtain analogues of **4**−**6** with reduced lipophilicity and improved aqueous solubility by replacement of these substituents by shorter alkyloxy-chains as well as phenoxy, benzyloxy, and phenyl residues.

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$_2\alpha$.[20,28] Because it is known that activated ketones can be metabolically unstable toward ketoreduction,[29−31] and because reduction of the ketone groups of our inhibitors to alcohols leads to inactive compounds,[31] we also tested the metabolic stability of all newly synthesized target compounds in a test system applying rat liver microsomes.

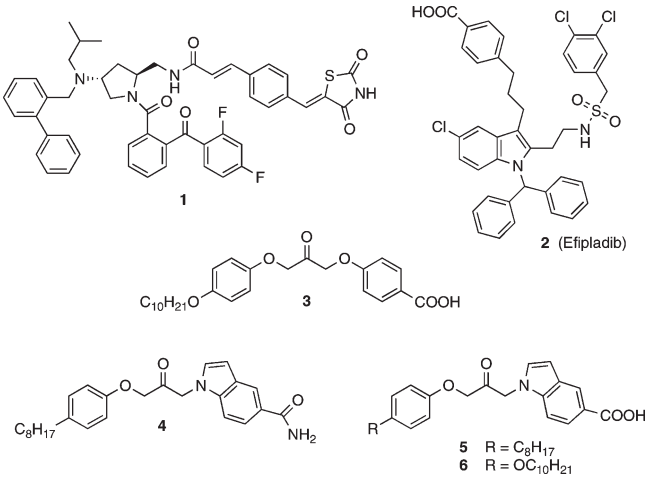


Figure 1

Furthermore, pharmacokinetic studies in mice were performed with one of the most promising cPLA$_2\alpha$ inhibitors synthesized, and finally this compound was tested in a topical in vivo model of inflammation.

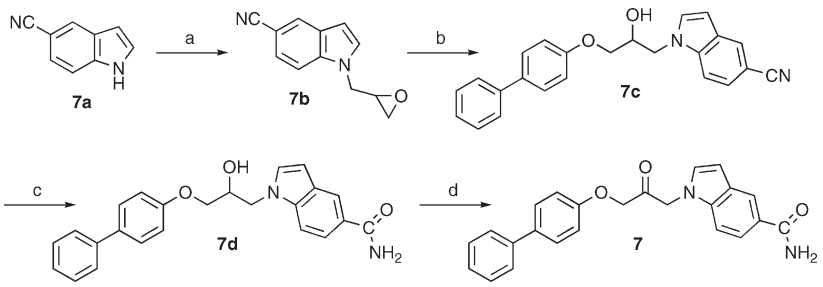
Chemistry

The indole-5-carboxamides **7**−**9** were prepared as exemplified for the *p*-biaryl derivative **7** (Scheme 1). Thus, indole-5-carbonitrile (**7a**) was treated with epichlororhydrin to afford the epoxy-intermediate **7b**. This compound was reacted with 4-phenylphenolate to generate the secondary alcohol **7c**. The nitrile moiety of **7c** was hydrolyzed by KOH in *tert*-butanol to afford the carboxamide **7d**. Finally, oxidation with acetic anhydride−DMSO gave rise to the target compound **7**.

During the synthesis of the lead compounds **5** and **6**, the carboxylic acid groups at indole 5 position were protected as *tert*-butyl esters.[28,32] In the last reaction step, the ester moieties were cleaved with trifluoroacetic acid. Because this procedure resulted in the formation of side products with a *tert*-butyl group in position 3 of the indole, we now decided to apply a benzyl ester protecting group instead. In some cases, the benzyl ester moiety was introduced in the course of the reaction sequence by transesterification, as outlined in Scheme 2 for the synthesis of **25**. Starting compound here was indole-5-carboxylic acid methyl ester (**25a**), which was alkylated in position 1 with epichlorohydrin to afford **25b**. Coupling of this compound with 4-octyloxyphenol in the presence of catalytic amounts of 4-dimethylaminopyridine without solvent provided hydroxy intermediate **25c**. Then the methyl ester of **25c** was converted to a benzyl ester group by reaction with potassium benzylate in benzyl alcohol. Oxidation of the secondary hydroxy group of obtained benzyl ester **25d** by acetic anhydride−DMSO and final cleavage of the ester group by catalytical hydrogenation gave the desired compound **25**.

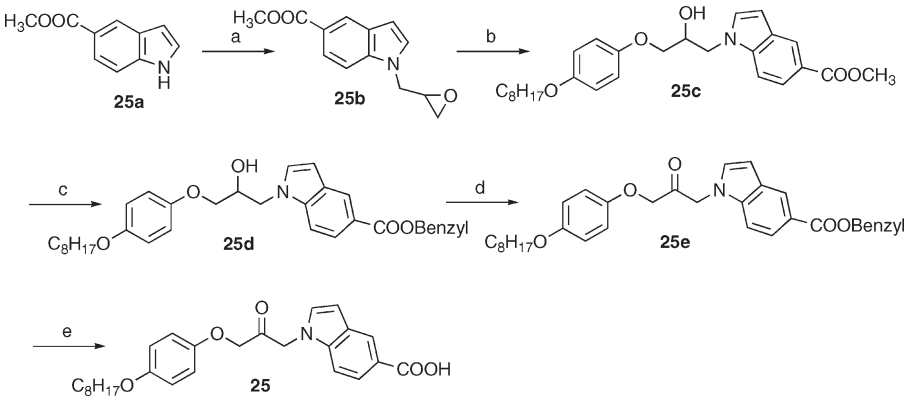
Alternatively, synthesis of several other target compounds, such as **10**, directly started from indole-5-carboxylic acid benzyl ester applying the same kind of reactions (Scheme 3). During hydrogenolytic cleavage of the benzylester protecting groups over Pd on charcoal, we did not observe a concomitant reduction of the ketone group like in the case of other activated ketones.[33] However, the indole heterocycle was reduced partly to an indoline. Because this side reaction could not be fully avoided by varying the reaction conditions, and because chromatographic separation of corresponding indole and indoline derivatives was difficult, the allyl ester moiety was chosen as alternative carboxylic acid protecting group. Scheme 4 illustrates this chemical approach by example of the synthesis of biaryl derivative **16**. Here, oxidation of the alcohol **16d** to the ketone **16e** was carried out with Dess−Martin

Scheme 1[a]



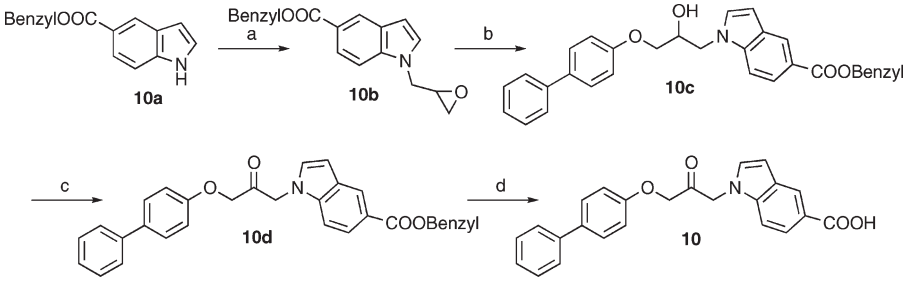
[a] (a) Epichlorohydrin, KOH, $Bu_4N^+Br^-$, room temp; (b) 4-phenylphenol, *tert*-BuLi, THF, reflux; (c) KOH, *tert*-butanol, 100 °C; (d) acetic anhydride, DMSO, room temp.

Scheme 2[a]



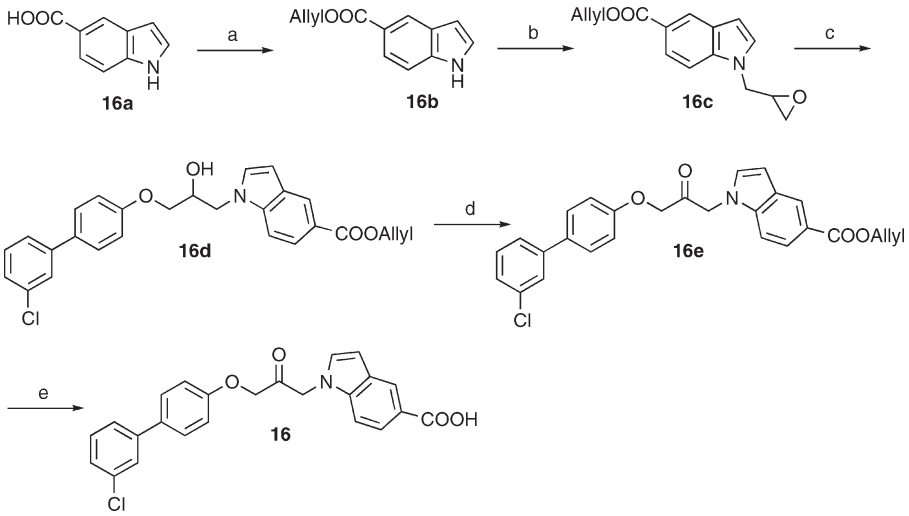
[a] (a) Epichlorohydrin, KOH, $Bu_4N^+Br^-$, room temp; (b) 4-octyloxyphenol, 4-dimethylaminopyridine, 90 °C; (c) benzyl alcohol, KH, 100 °C; (d) acetic anhydride, DMSO, room temp; (e) H_2, Pd/C, THF, room temp.

Scheme 3[a]



[a] (a) Epichlorohydrin, KOH, $Bu_4N^+Br^-$, room temp; (b) 4-phenylphenol, 4-dimethylaminopyridine, 90 °C; (c) acetic anhydride, DMSO, room temp; (d) H_2, Pd/C, THF, room temp.

Scheme 4[a]



[a] (a) Allyl bromide, $NaHCO_3$, DMF, room temp; (b) epichlorohydrin, KOH, $Bu_4N^+Br^-$, room temp; (c) 4-(3-chlorophenyl)phenol, 4-dimethylaminopyridine, 90 °C; (d) Dess−Martin periodinane reagent, CH_2Cl_2, room temp; (e) $Pd(PPh_3)_4$, CH_3COOH, THF, room temp.

periodinane reagent. Cleavage of the allyl ester of **16e** was achieved by Pd(0)-catalyzed allyl transfer.

The preparation of the 3-acetylated indole derivatives **34** and **35** (Figure 2) was also performed via this route starting from 3-acetylindole-5-carboxylic acid allyl ester. The 3-acetylindole **33** was afforded using 3-acetylindole-5-carboxylic acid benzyl ester as educt, applying the synthetic approach described for the synthesis of **10**.

Scheme 5 outlines the synthesis of the 3-isobutanoylindole-5-carboxylic acid derivative **36**. Indole-5-carboxylic acid methyl ester was acylated in position 3 with isobutanoyl chloride to provide **36a**. Introduction of the 2-oxo-3-(4-phenoxyphenoxy)propyl residue in position 1 of this indole compound was carried out in a fashion similar to that described for the synthesis of **16e** (Scheme 4). Next, the ketone moiety of obtained intermediate **36d** was acetalized with

orthoformic acid triethylester in the presence of catalytic amounts of H_2SO_4. The methyl ester group **36d** was saponified with aqueous NaOH. Finally, the acetal protecting group was removed with aqueous HCl in THF to yield the target compound **36**. The alcohol derivative **37** of compound **36** was obtained by alkaline ester hydrolysis of **36c**.

Evaluation of the Target Compounds

Inhibition of cPLA$_2\alpha$. The target compounds were evaluated in an assay applying cPLA$_2\alpha$ isolated from human platelets.[34] Enzyme activity was measured in a solution containing covesicles of 1-stearoyl-2-arachidonoyl-*sn*-glycero-3-phosphocholine (SAPC) and 1,2-dioleoyl-*sn*-glycerol (DOG). Inhibitory potency of the test compounds was assessed by comparing the amount of arachidonic acid released from SAPC in their absence and presence after an incubation time of 60 min with reversed phase HPLC and UV detection at 200 nm.

Compound **36** was also tested in cellular situation. In this assay, cPLA$_2\alpha$ of intact human platelets was activated with the phorbol ester 12-*O*-tetradecanoylphorbol-13-acetate (TPA).[35] The cPLA$_2\alpha$-catalyzed liberation of arachidonic acid from membrane phospholipids was measured after 60 min with reversed phase HPLC and UV detection at 200 nm. To avoid metabolism of arachidonic acid via the cyclooxygenase-1 and the 12-lipoxygenase pathways, the dual cyclooxygenase/12-lipoxygenase inhibitor 5,8,11,14-eicosatetraynoic acid (ETYA) was added to the platelets in these experiments.

Metabolic Stability. Test compounds were incubated with rat liver microsomes under aerobic conditions in absence and presence of the cofactor NADPH as described previously.[35] The metabolic reactions were terminated after 30 min. The extent of metabolism was evaluated with reversed phase HPLC and UV detection. The structures of the main metabolites were confirmed by HPLC/MS analysis.

Water Solubility. Water solubility was determined experimentally according to published procedures.[35,36] Briefly, sodium phosphate buffer (pH 7.4) was added to a test compound, and the suspension obtained was equilibrated by sonication (10 min) in a bath sonicator and shaking at room temperature (20 h), followed by centrifugation. An aliquot of the supernatant was diluted with acetonitrile, and the concentration of the test compound was determined by reversed phase HPLC and UV-detection using a standard curve.

Partition Coefficient. The partition coefficients (log *P*) of the target compounds were determined by reversed phase HPLC according to the published OECD procedure.[37]

Bioavailability. Compound **36** was administered to C57Bl6 mice either perorally or intravenously. From each animal, venous blood was withdrawn twice at different times. The plasma samples obtained by centrifugation were diluted with phosphate buffered saline (pH 7.4) and spiked with internal standard. After precipitation of proteins by addition of acetonitrile, the samples were centrifuged and the concentrations of **36** and its alcohol metabolite **37** were determined by HPLC and UV detection. In the same way, plasma

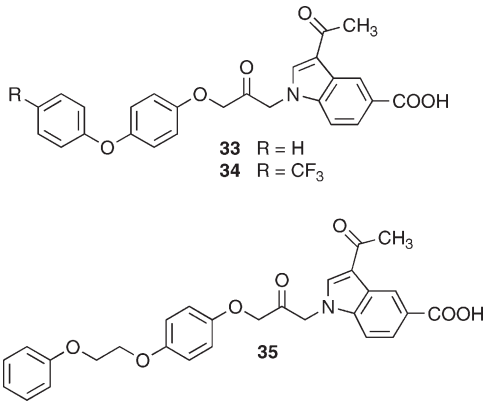
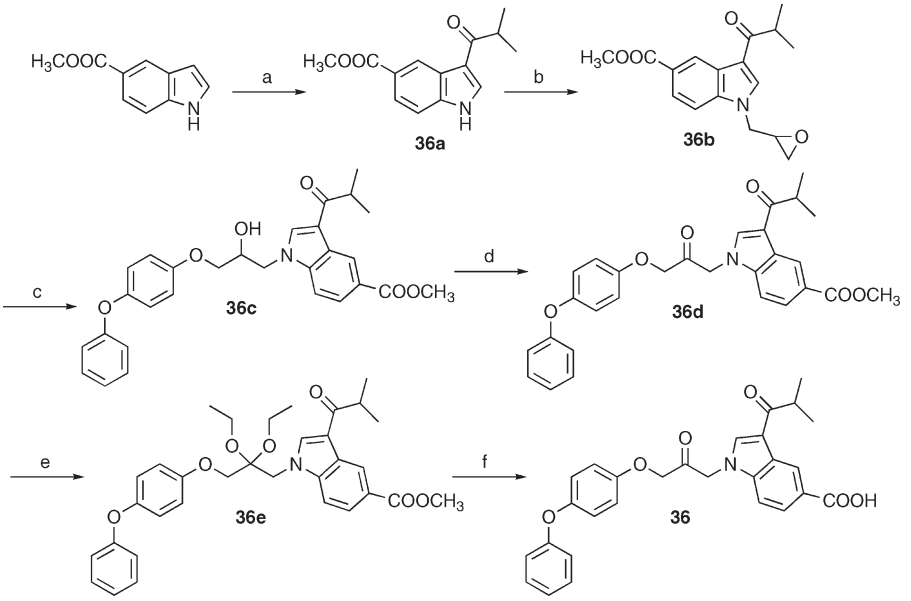


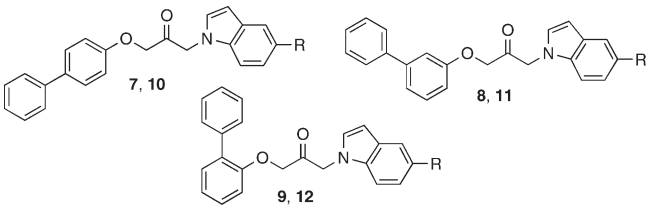
Figure 2

Scheme 5[a]



[a] (a) (1) $ZnCl_2$, BuLi, CH_2Cl_2, room temp, (2) isobutanoyl chloride, $AlCl_3$, 0 °C−room temp; (b) epichlorohydrin, KOH, $Bu_4N^+Br^-$, room temp; (c) 4-phenoxyphenol, 4-dimethylaminopyridine, 120 °C; (d) Dess−Martin periodinane reagent, CH_2Cl_2, room temp; (e) orthoformic acid triethylester, ethanol, H_2SO_4, reflux; (d) (1) aq NaOH, methanol, reflux, (2) aq HCl, THF, reflux.

Table 1. cPLA$_2$α-Inhibitory Potency, Metabolic Stability and Thermodynamic Solubility of Indole-5-carboxamide and Indole-5-carboxylic Acid Derivatives



compd	R	inhibition of cPLA$_2$α IC$_{50}$ (μM)[a]	metabolic stability (%)[b]	thermodynamic solubility (μg/mL)[c]	log P[d]
7	$CONH_2$	0.15	33	< 3	2.4
8	$CONH_2$	0.25	15	< 3	2.3
9	$CONH_2$	na[e]	20	< 3	2.3
10	COOH	0.064	7	5 ± 1	3.2
11	COOH	0.21	13	16 ± 3	3.1
12	COOH	na[e]	81	21 ± 2	3.1
4		0.12	54	< 3	5.5
5		0.035	65 ± 5	< 3	6.3
6		0.020	67	< 3	7.1

[a] Values are the means of at least two independent determinations, errors are within ±20%; IC$_{50}$ value of reference inhibitor **3**: 0.011 μM.[20,28] [b] Percentage of parent compound remaining after metabolism by rat liver microsomes; values are the means of at least two independent determinations; in case of **5**: mean ± standard deviation, $n = 5$. [c] Mean ± standard deviation, $n = 3$, in case of values < 3 μg/mL: $n = 2$; solubility of reference indomethacin: 246 ± 18 μg/mL ($n = 4$). [d] Partition coefficient (log P) determined by reversed phase HPLC; log P of reference indomethacin: 2.9. [e] na: not active at 1 μM.

concentrations of the reference indomethacin after po application were determined.

Ear Edema Model of Contact Dermatitis. Topical anti-inflammatory effects of **36** were evaluated in a murine model of acute irritant contact dermatitis.[38,39] Ear inflammation was induced by treating the dorsal surface of the right ear with 5% benzalkonium chloride solution. After 10 min, test substance dissolved in acetone, or acetone alone (control) was applied on the benzalkonium chloride treated ear. Ear swelling of the right ear was measured over 24 h and compared with the thickness of the vehicle treated left ear.

Results and Discussion

In previous investigations, we have found that the indole-5-carboxylic acid **5** and its carboxamide derivative **4** are potent inhibitors of cPLA$_2$α (IC$_{50}$: 0.035 and 0.12 μM, respectively). To reduce the high lipophilicity of these compounds, we now wanted to replace the 4-octylphenoxy group by a 4-phenylphenoxy residue. Furthermore, we planned to synthesize the isomeric 3-phenylphenoxy and 2-phenylphenoxy-derivatives. Because the phenylphenoxy substituent is very rigid, we hoped to get additional information about the steric requirements for inhibitor binding by this structural variation. First, the appropriate indole-5-carboxamides were synthesized because their synthesis seemed to be more straightforward than that of the corresponding acids. With an IC$_{50}$ of 0.15 μM, the 4-phenylphenoxy-substituted compound **7** inhibited cPLA$_2$α with about the same potency as the lead **4** (Table 1). Shifting the position of the terminal phenyl substituent of the phenoxy group reduced inhibitory activity. While the 3-phenylphenoxy derivative **8** still had a considerable activity (IC$_{50}$: 0.25 μM), the indole with a 2-phenylphenoxy group (**9**) was totally inactive at a concentration of 1 μM.

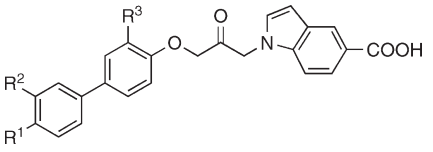
Reduction of the lipophilicity of **4** by replacement of octyl by phenyl, reflected by a decrease of the log P value from 5.5 (**4**) to 2.4 (**7**), led to a decrease of metabolic stability (54% vs 33%). As in the case of indole-5-carboxylic acid **5**, the main metabolic reaction in rat liver microsomes was the reduction of the ketone moiety of **4** and **7**, respectively, to a secondary alcohol as shown by HPLC-MS experiments. In contrast, water solubility was not significantly affected by this structural modification. The amount of **7** dissolved under the conditions applied was still less than 3 μg/mL.

Hoping to get better soluble compounds than the amides **7**−**9**, we synthesized the corresponding carboxylic acid derivatives **10**−**12**. Analogously to the results seen for the amides, the 4-phenyl-substituted derivative (**10**) possessed the highest cPLA$_2$α inhibitory potency of these derivatives (Table 1). With an IC$_{50}$ of 0.064 μM, **10** was about half as active as the 4-octyl substituted lead **5**. The inactivity of the 2-phenylphenoxy derivative **12** at the highest test concentration (1 μM) showed again that shifting the terminal phenyl substituent from 4- to 2-position of the phenoxy residue is not tolerated by the enzyme. While the amides **7**−**9** had about the same metabolic stability, the 2-phenyl-substituted indole-5-carboxylic acid derivative **12** was significantly more stable against keto reduction than its 3-phenyl and 4-phenyl isomers **10** and **11**. With a value of 21 μg/mL, **12** also possessed the highest water solubility of this series. However, the solubilities of **12** as well as of **10** (5 μg/mL) and **11** (16 μg/mL) still lay under the limit of 50 μg/mL, which is estimated to be necessary for a sufficient po bioavailability of drugs with midrange permeability and average potency.[40]

Next, we investigated the effect of the introduction of diverse substituents in the biaryl part of indole-5-carboxylic acid **10**. Substitution of the 4′-position with a fluorine atom (**13**) or with the lipophilic substituents chloro, methyl, and isopropyl (**15**, **17**−**18**) increased cPLA$_2$α inhibitory potency (about 1.5- to 3-fold) and metabolic stability (Table 2). The same effects were achieved with fluoro- and chloro-atoms in 3′-position of the biaryl system (**14**, **16**). On the contrary, a polar methoxy group in 4′-position (**19**) slightly lowered enzyme inhibition and metabolic stability. With solubility values lower than 3 μg/mL all 3′- and 4′-substituted biaryl compounds (**13**−**19**) were less water-soluble than the unsubstituted parent compound **10** (5 μg/mL).

Introduction of substituents in *ortho*-position of the phenoxy residue resulted in a marked fall of cPLA$_2$α inhibitory potency. With an IC$_{50}$ value of 0.23 μM the fluoro-substituted compound **20** was about three times less active than the unsubstituted compound **10**. Chloro and methyl substituents (**21**, **22**) reduced enzyme inhibition at 1 μM to a value less than 50%. The methoxy-substituted derivative **22** was inactive at this concentration. The published 1,3-diaryloxypropan-2-one **3**[20] was reported to form covalent bonds with the serine of the active site of cPLA$_2$α via its activated electrophilic ketone moiety. Because our indole-5-carboxylic acids with 3-aryloxy-2-oxopropyl groups in position 1 are structurally related to that inhibitor, they obviously possess such a serine trap too. Probably subsituents in the *ortho*-position of the phenoxy residue sterically hinder the attack of the active site serine to the activated ketone, thus lowering enzyme inhibition. The accessibility of metabolizing enzymes of rat liver microsomes to the ketone seems to be impeded, too, because metabolic reduction declines with the introduction of these substituents.

Table 2. cPLA$_2$α-Inhibitory Potency, Metabolic Stability and Thermodynamic Solubility of Indole-5-carboxylic Acid Derivatives



compd	R^1	R^2	R^3	inhibition of cPLA$_2$α IC_{50} (μM)[a]	metabolic stability (%)[b]	thermodynamic solubility (μg/mL)[c]	log P[d]
10	H	H	H	0.064	7	5 ± 1	3.2
13	F	H	H	0.037	14	< 3	3.2
14	H	F	H	0.041	22	< 3	3.2
15	Cl	H	H	0.027	46	< 3	3.9
16	H	Cl	H	0.019	31	< 3	3.8
17	CH_3	H	H	0.040	22	< 3	3.7
18	$CH(CH_3)_2$	H	H	0.035	40	< 3	4.7
19	OCH_3	H	H	0.090	2	< 3	3.0
20	H	H	F	0.23	16	5 ± 0.3	3.3
21	H	H	Cl	> 1[e]	61	< 3	3.7
22	H	H	CH_3	> 1[f]	33	< 3	3.8
23	H	H	OCH_3	na[g]	72	6 ± 2	2.9

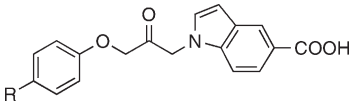
[a] Values are the means of at least two independent determinations, errors are within ±20%. [b] Percentage of parent compound remaining after metabolism by rat liver microsomes; values are the means of at least two independent determinations. [c] Mean ± standard deviation, $n = 3$, in case of values < 3 μg/mL: $n = 2$. [d] Partition coefficient (log P) determined by reversed phase HPLC. [e] 29% inhibition at 1 μM. [f] 16% inhibition at 1 μM. [g] na: not active at 1 μM.

One of the most active inhibitors reported in our recent publication[28] was the 4-decyloxy substituted compound **6** (IC_{50}: 0.020 μM). We now synthesized derivatives of **6** with reduced lipophilicity by shortening the alkoxy group. Stepwise reduction of this chain from decyloxy to hexyloxy led to a loss of activity (Table 3). However, this decrease was not very pronounced because the hexyloxy-substituted derivative **27** still had a considerable activity (IC_{50}: 0.034 μM). From all alkoxy compounds, the octyloxy derivative **25** possessed the highest metabolic stability (79%). The main metabolite here was its corresponding secondary alcohol as shown by HPLC/MS experiments. In the case of the elongated decyloxy compound **6**, the amount of alcohol produced was even smaller. However, concomitantly, considerable amounts of more polar metabolites of unknown type were formed here so that less parent compound (67%) could be measured. From the alkoxy derivatives investigated, the most polar derivative **27** (log P: 4.6) possessed the lowest metabolic stability (38%) due to a high rate of keto reduction. On the other hand, the aqueous solubility of **27** (7 μg/mL) was higher than that of its more lipophilic homologues (< 3 μg/mL).

Replacement of the hexyloxy residue by a phenoxy group did not change cPLA$_2$α inhibitory potency, while polarity (log P: 3.1) and aqueous solubility (43 μg/mL) of the phenoxy-substituted compound **28** were significantly higher than that of the hexyloxy derivative **27**. The increased polarity, however, again resulted in a drastic drop of metabolic stability. While still 38% of **27** remained after the metabolic reactions, only 3% of **28** could be detected under the same conditions. Introduction of a trifluoromethyl group in position 4 of the phenoxy group of **27** elevates enzyme inhibitory potency and metabolic stability but reduces aqueous solubility (< 3 μg/mL) and lipophilicity (log P: 4.0) of the obtained compound **29**.

Interestingly, the two indole-5-carboxylic acid derivatives with terminal benzyl groups (**30** and **31**) had about the same inhibitory activity against cPLA$_2$α, the same metabolic stability, and the same aqueous solubility although their log P values were significantly different (3.3 vs 4.1). Thus, contrary to the trifluoromethylation of **28** at the terminal phenyl residue, the elevation of lipophilicity of **30** produced by introduction of two methyl groups at the CH_2-part of the benzyl residue did not significantly affect the investigated properties.

Table 3. cPLA$_2$α-Inhibitory Potency, Metabolic Stability and Thermodynamic Solubility of Indole-5-carboxylic Acid Derivatives



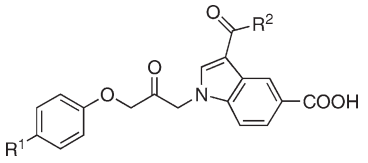
compd	R	inhibition of cPLA$_2$α IC_{50} (μM)[a]	metabolic stability (%)[b]	thermodynamic-solubility (μg/mL)[c]	log P[d]
6	$OC_{10}H_{21}$	0.020	67	< 3	7.1
24	OC_9H_{19}	0.023	74	< 3	6.5
25	OC_8H_{17}	0.026	79	< 3	5.9
26	OC_7H_{15}	0.032	57	< 3	5.2
27	OC_6H_{13}	0.034	38	7 ± 1	4.6
28	O-phenyl	0.036	3	43 ± 8	3.1
29	O-(4-CF_3-phenyl)	0.019	57	< 3	4.0
30	CH_2-phenyl	0.054	7	18 ± 3	3.3
31	$C(CH_3)_2$-phenyl	0.047	0	25 ± 1	4.1
32	OCH_2CH_2O-phenyl	0.11	21	31 ± 3	3.1

[a] Values are the means of at least two independent determinations; errors are within ±20%. [b] Percentage of parent compound remaining after metabolism by rat liver microsomes; values are the means of at least two independent determinations. [c] Mean ± standard deviation, $n = 3$; in case of values < 3 μg/mL: $n = 2$. [d] Partition coefficient (log P) determined by reversed phase HPLC.

Next we replaced the oxygen- and CH_2-spacer, respectively, between the two phenyl residues of **28** and **30** by a longer polar ethylendioxy spacer. This structural variation led to a 2- to 3-fold reduction of enzyme inhibition in combination with a slight increase of metabolic stability. Aqueous solubility of the obtained compound **32** lay between those of **28** and **30**.

Recently, we have found that introduction of an acetyl moiety at indole-3-position of the lead **5** raised cPLA$_2$α

Table 4. cPLA$_2$α-Inhibitory Potency, Metabolic Stability and Thermodynamic Solubility of Indole-5-carboxylic Acid Derivatives



compd	R^1	R^2	inhibition of cPLA$_2$α IC_{50} (μM)[a]	metabolic stability (%)[b]	thermodynamic solubility (μg/mL)[c]	log P[d]
33	O-phenyl	CH_3	0.012	41	111 ± 19	2.6
34	O-(4-CF_3-phenyl)	CH_3	0.010	73	< 3	3.5
35	OCH_2CH_2O-phenyl	CH_3	0.041	68	34 ± 2	2.6
36	O-phenyl	$CH(CH_3)_2$	0.012	30	212 ± 15	3.5

[a] Values are the means of at least two independent determinations, errors are within ±20%; IC_{50}-value of reference inhibitor **3**: 0.011 μM.[20,28] [b] Percentage of parent compound remaining after metabolism by rat liver microsomes; values are the means of at least two independent determinations; [c] Mean ± standard deviation, $n = 3$, in case of values < 3 μg/mL: $n = 2$; solubility of reference indomethacin: 246 ± 18 μg/mL ($n = 4$). [d] Partition coefficient (log P) determined by reversed phase HPLC; log P of reference indomethacin: 2.9.

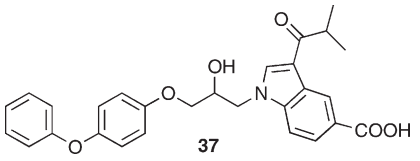


Figure 3. Chemical structure of the alcohol metabolite of **36**.

inhibitory potency.[28] Therefore, we also synthesized analogous acetyl derivatives of **28**, **29**, and **32**. As shown in Table 4, this structural modification not only increased enzyme inhibitory activity of the compounds 2- to 3-fold but also improved their metabolic stability. The elevated polarity of the obtained compounds **33**−**35**, reflected by lowered log P values, only led to better aqueous solubility in case of the phenoxy-substituted derivative **33** (**28**: 43 μg/mL; **33**: 111 μg/mL). The solubility of the acetylated indoles **34** and **35** was not significantly higher than that of their parent compounds **29** and **32**.

Moreover, we synthesized a derivative of **33** with a slightly longer but branched acyl residue at indole 3 position. The obtained 3-isobutanoylindole-5-carboxylic acid **36** was as active as the 3-acetyl-derivative **33** (IC_{50}: 0.012 μM) and somewhat less stable against metabolic reduction to the alcohol **37** (Figure 3). Although polarity of **36** was significantly lower than that of **33** (log P: 3.5 vs 2.6), with an aqueous solubility of about 210 μg/mL **36** was about 2-fold better soluble than **33**. These results show again, that the lipophilicity/polarity-data of the compounds expressed by their log P values, are not a good predictor for aqueous solubility in our case. Other structural features, such like chain-branching, can have a large influence on this property.

Additionally, compound **36** was tested for cPLA$_2$α inhibition in a cellular situation. In intact human platelets cPLA$_2$α activity triggered by phorbol ester TPA was also inhibited with high potency (IC_{50} of 0.015 ± 0.002 μM, $n = 3$).

To evaluate selectivity of **36** for inhibition of cPLA$_2$α, inhibitory potency of this compound against calcium-independent PLA$_2$ (iPLA$_2$) from rat brain cytosol as well as group IB secretory PLA$_2$ (sPLA$_2$) from porcine pancreas was determined.[41,42] At a concentration of 10 μM, compound **36** did not affect activity of these two PLA$_2$ enzymes. In contrast, at this concentration, the iPLA$_2$ inhibitor bromoenol lactone produced an about 50% inhibition of iPLA$_2$ activity, and the sPLA$_2$ inhibitor (*S*)-5-(4-benzyloxyphenyl)-4-[(7-phenylheptanoyl)amino]pentanoic acid[43] blocked activity of group IB sPLA$_2$ by 89% ($n = 2$) (see Supporting Information for more information).

Furthermore, pharmacokinetic studies were performed with compound **36**. Plasma levels of **36** and its alcohol metabolite **37** in mice were determined by HPLC and UV-detection after iv administration of 10 mg/kg **36** dissolved in DMSO/phosphate-buffered saline (pH 7.4) and after po administration of 100 mg/kg **36** suspended in aqueous methyl cellulose solution. As reference, 10 mg/kg indomethacin was applied perorally in the same way. After iv injection, from each mouse blood samples were collected after 5 and 30 min. In the experiments with po dosage, blood was withdrawn from the animals after 1 and 2 h. Table 5 summarizes the results obtained.

The data show that, after iv administration of **36** (10 mg/kg), an extremely rapid plasma clearance took place. From about 250 μg **36** injected initially into the mice (blood volume about 2 mL), only about 40 μg still could be found in the plasma after 5 min in the form of the parent compound **36** and its main phase I-metabolite **37**. After 30 min, even less than 1 μg/mL of **36** and **37** were present. After po application of 100 mg/kg **36** also very low plasma levels of **36** and **37** were measured after 1 h (about 5 μg/μL) and 2 h (< 1 mg/μL).

In our opinion, the low po bioavailability is not due to an unfavorable lipophilicity or solubility of **36** because the reference indomethacin, which possesses a similar lipophilicity (log P: 2.9 vs 3.5) as well as aqueous solubility (246 vs 212 μg/mL), gave high plasma levels after 1 and 2 h, respectively, even when only 10 mg/kg were applicated perorally. An impaired cellular permeability is also not likely to be the reason for the low plasma levels because **36** showed high cPLA$_2$α inhibitory activity in a cellular assay too. In fact, the high plasma clearance of **36** seems to be the main cause for the low po bioavailability. This efficient clearance may be due to an excessive glucuronidation of **36** and **37** in combination with a high biliar excretion. A hint for this was the high level of free and bound **36** and **37** found in the bile of the animals. The biliar concentration 30 min after iv administration of 10 mg/kg **36** ranged up to 200 μg/mL of **36** and 96 μg/mL of **37**. After treatment with glucuronidase, these concentrations increased up to 740 and 1390 μg/mL, respectively. Similar results were observed 2 h after po dosage (100 mg/kg). Before incubation

Table 5. Plasma Levels of the cPLA$_2\alpha$ Inhibitor **36** and Its Alcohol Metabolite **37** after iv or po Administration of **36** to Mice, And Plasma Levels of the Reference Indomethacin after po Dosage

		plasma levels (μg/mL)[a]	
dosage	time	**36**	**37**
compd **36**			
10 mg/kg iv	5 min	15 ± 8	6.2 ± 2.9
	30 min	0.4 ± 0.2	0.5 ± 0.3
100 mg/kg po	1 h	3.0 ± 2.4	1.7 ± 1.9
	2 h	0.4 ± 0.3	0.3 ± 0.2
indomethacin			
10 mg/kg po	1 h	42 ± 7.2	
	2 h	34 ± 5.3	

[a] Values are means ± standard deviations; compound **36**: $n = 3$; indomethacin: $n = 4$.

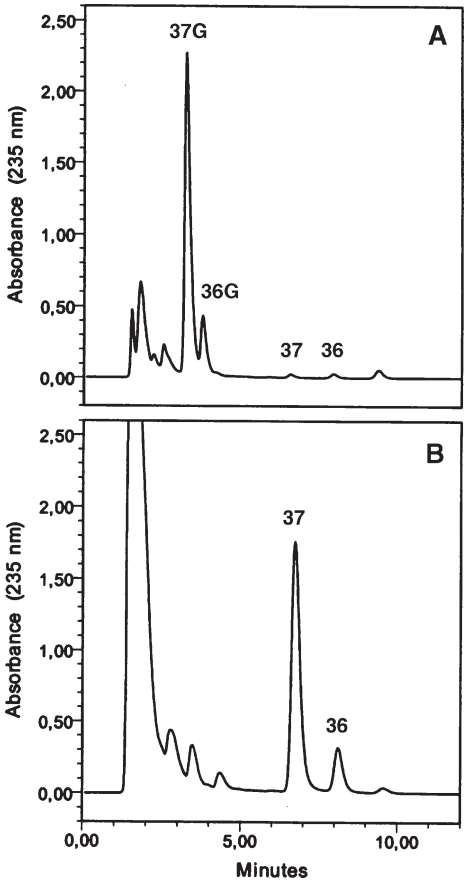


Figure 4. Reversed phase HPLC-UV chromatograms of bile samples of a mouse perorally dosed with 100 mg/kg of **36** before (A) and after (B) treatment with glucuronidase. Column: Phenomenex Aqua 3 μ C_{18} column (4.6 mm I.D. × 100 mm); solvent: acetonitrile/water/phosphoric acid (85%) (60:40:0.1, v/v/v); flow: 0.7 mL/min; UV detection: 235 nm. **36G**: glucuronide of **36**; **37G**: glucuronide of **37**. Concentrations in the bile samples before treatment with glucuronidase: 104 μg/mL of **36** and 42 μg/mL of **37**; after treatment with glucuronidase: 1480 μg/mL of **36** and 4970 μg/mL of **37**.

with glucuronidase, the biliar concentration levels of **36** and **37** amounted up to 104 and 42 μg/mL, while thereafter these values rose to maximal values of 1480 μg/mL (**36**) and 4970 μg/mL (**37**), respectively. Figure 4 shows the HPLC/UV chromatograms of bile samples of a perorally dosed mouse before and after treatment with glucuronidase. The presence of the glucuronides of **36** and **37** was confirmed by high resolution mass spectrometry (see Supporting Information for more information).

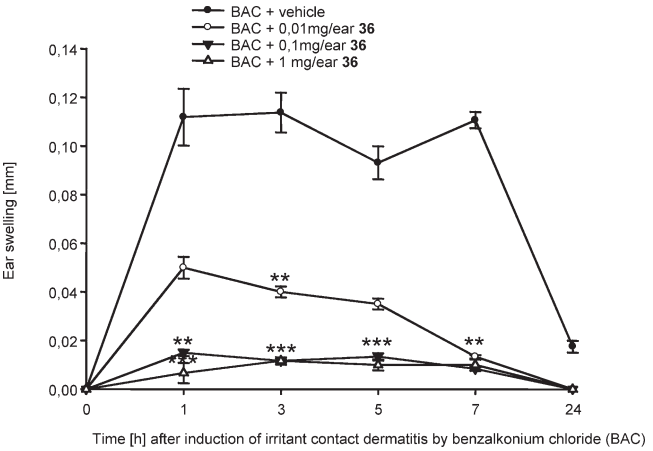


Figure 5. Dose-dependent effects of **36** in a murine model of irritative contact dermatitis. Ear thickness was measured after benzalkonium chloride (5%)-induced ear inflammation in BALB/c mice post-treated with an acetone solution of **36** (0.01, 0.1, and 1.0 mg/ear) or acetone alone (vehicle). $N = 5$ per group. Each value represents mean ± standard deviation; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Because of the data obtained in the pharmacokinetic studies, it did not make sense to evaluate **36** in a systemic animal model of inflammation, such as the carrageenan induced rat paw model. However, testing the compound for topical anti-inflammatory effects seemed to be reasonable. Therefore, finally **36** was investigated in a murine model of acute irritant contact dermatitis.[38,39] Skin inflammation was induced by benzalkonium chloride. After 10 min, the drug was applied topically at three different amounts (0.01, 0.1, and 1.0 mg/ear). Ear swelling was measured over 24 h. Edema formation was significantly reduced by **36** as early as the first hour after the induction of dermatitis, and this reduction was still significant 5 and 7 h, respectively, later (Figure 5). While the doses of 0.1 and 1.0 mg/ear produced similar potent effects, ear swelling was less reduced by the lowest dose of 0.01 mg/ear, reflecting the dose dependency of the effect. In a second independent experiment, the anti-inflammatory effect of **36** in the acute irritant contact dermatitis model at a dose of 0.1 mg/ear was compared with the effect of the potent glucocorticoid clobetasol-17-propionate at a dose of 0.005 mg/ear. At the peak of edema formation, which appeared 7 h after application of the irritant benzalkonium chloride, **36** produced similar anti-inflammatory effects as the glucocorticoid. Inhibition of ear swelling by **36** was 84 ± 6%, while clobetasol-17-propionate reduced edema formation by 90 ± 5% (means ± standard deviations, $n = 15$). These results suggest that **36** is useful for the treatment of skin inflammation.

In summary, we have developed novel derivatives of the cPLA$_2\alpha$ inhibitor **5** with reduced lipophilicity, improved aqueous solubility, and still high enzyme inhibitory potency, such as compound **36**. While oral bioavailability of **36** in mice was not favorable, it showed strong anti-inflammatory effects when applied topically in a murine irritant ear edema model. Further evaluation of this class of inhibitors and structural refinements of **36** to reduce its excessive glucuronidation are in progress and 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) and electrospray ionization (ESI), respectively. The purity of all target compounds was determined using two diverse HPLC systems with UV-detection at 254 nm. The first one applied an amino phase (Spherisorb NH_2, 5 μm, 4.0 mm I.D. × 250 mm, Latek, Heidelberg, Germany) eluting the compounds with an isohexane/THF gradient at a flow rate of 0.75 mL/min. In the second system, separation was performed using a cyano phase (LiChrospher 100 CN, 5 μm, 3.0 mm I.D. × 250 mm, Merck, Darmstadt, Germany) with an isohexane/THF gradient containing 0.1% trifluoroacetic acid at a flow rate of 0.5 mL/min. Purity of most of the 30 target compounds was ≥95%. Purities of **8**, **10**, **18**, **24**, **29**, and **30** lay between 91% and 94% (for detailed data see Supporting Information).

1-Oxiranylmethylindole-5-carbonitrile (7b). A mixture of powdered KOH (88%; 2.2 g, 34 mmol), indole-5-carbonitrile (**7a**) (3.0 g, 21 mmol), tetrabutylammonium bromide (0.68 g, 2.1 mmol), and epichlorohydrin (10 mL) was stirred at room temperature overnight. After addition of water, the mixture was exhaustively extracted with ethyl acetate. The combined organic phases were washed with water three times and dried (Na_2SO_4). The solvent was distilled off and the residue purified by chromatography on silica gel (hexane/ethyl acetate, 7:3) to give **7b** as a solid (3.3 g, 79%); mp 92−93 °C. ^{1}H NMR ($CDCl_3$): δ 2.45 (dd, 1H), 2.83−2.85 (m, 1H), 3.27−3.30 (m, 1H), 4.15 (dd, 1H), 4.54 (dd, 1H), 6.61 (d, 1H), 7.26−7.27 (m, 1H), 7.44−7.45 (m, 2H), 7.97 (s, 1H). MS (EI) *m*/*z* (%) 198 (75) [M]$^+$, 142 (100).

1-[3-(Biphenyl-4-yloxy)-2-hydroxypropyl]indole-5-carbonitrile (7c). Under a nitrogen atmosphere, a solution of 4-phenylphenol (0.34 g, 2.0 mmol) in dry THF (5 mL) was treated under vigorous stirring dropwise with a solution of *tert*-butyllithium (1.0 mmol) in pentane. After 5 min, a solution of **7b** (0.20 g, 1.0 mmol) in dry THF (5 mL) was added. The mixture was heated under reflux for 24 h, cooled, and diluted with diethyl ether. The organic mixture was washed three times with water, and the aqueous phases were re-extracted with ethyl acetate. The combined organic phases were dried, and the solvent was evaporated. The residue was chromatographed on silica gel (hexane/ethyl acetate, 7:3) to yield **7c** as a solid (0.38 g, 98%); mp 133 °C. ^{1}H NMR (DMSO-d_6): δ 3.90−3.92 (m, 2H), 4.20 (m, 1H), 4.27 (dd, 1H), 4.42 (dd, 1H), 5.45 (d, 1H), 6.60 (d, 1H), 7.04 (d, 2H), 7.30 (d, 1H), 7.40 (d, 2H), 7.45 (d, 2H), 7.58 (d, 1H), 7.59 (d, 1H), 7.62 (m, 2H), 7.72 (d, 1H), 8.35 (s, 1H). MS (EI): *m*/*z* (%) 368 (1) [M$^+$], 155 (100).

1-[3-(Biphenyl-4-yloxy)-2-hydroxypropyl]indole-5-carboxamide (7d). A mixture of **7c** (0.20 g, 0.54 mmol), *tert*-butanol (10 mL), and powdered KOH (88%; 0.27 g, 4.2 mmol) was heated under reflux for 2 h. After cooling, water was added and the reaction mixture was neutralized with 1 M HCl. Then the mixture was extracted exhaustively with ethyl acetate. The combined organic phases were washed with water and brine and dried (Na_2SO_4). The solvent was evaporated and the residue purified by silica gel chromatography eluting with ethyl acetate to yield **7d** as a solid (88 mg, 42%); mp 155 °C. ^{1}H NMR (DMSO-d_6): δ 3.88−3.92 (m, 2H), 4.19 (s, br, 1H), 4.27 (dd, 1H), 4.41 (dd, 1H), 5.48 (s, br, 1H), 6.51 (d, 1H), 7.02 (d, 2H), 7.10 (s, br, 1H), 7.28−7.32 (m, 1H), 7.36−7.43 (m, 3H), 7.48−7.63 (m, 6H), 7.83 (s, br, 1H), 8.17 (s, 1H). MS(EI): *m*/*z* (%) 386 (42) [M$^+$], 173 (100).

1-[3-(Biphenyl-4-yloxy)-2-oxopropyl]indole-5-carboxamide (7). Acetic anhydride (0.6 mL, 6.4 mmol) was added to dry DMSO (5 mL), and the mixture was stirred under a nitrogen atmosphere at room temperature for 10 min. Then this solution was added to a solution of **7d** (60 mg, 0.16 mmol) in dry DMSO (5 mL). The mixture was stirred under a nitrogen atmosphere for 18 h, poured into a mixture of 5% aqueous $NaHCO_3$ and brine (1:1), and extracted exhaustively with ethyl acetate. The combined organic phases were washed three times with half-saturated brine, dried (Na_2SO_4), and the solvent was distilled off. The residue recrystallized from hexane/ethylacetate to yield **7** as a solid (60 mg, 98%); mp 120 °C. ^{1}H NMR (DMSO-d_6): δ 5.09 (s, 2H), 5.21 (s, 2H), 6.57 (d, 1H), 7.06 (d, 2H), 7.11 (s, broad, 1H), 7.28−7.33 (m, 1H), 7.35 (d, 1H), 7.38−7.45 (m, 3H), 7.57−7.70 (m, 5H), 7.85 (s, broad, 1H), 8.17 (s, 1H). MS (EI): *m*/*z* (%) 384 (31) [M$^+$], 173 (100).

Benzyl 1-Oxiranylmethylindole-5-carboxylate (10b). Compound **10b** was synthesized from benzyl indole-5-carboxylate[44] (**10a**) (0.60 g, 2.4 mmol) according to the procedure described for the preparation of **25b**. Yield: 0.51 g (69%); mp 66−67 °C. ^{1}H NMR ($CDCl_3$): δ 2.44 (dd, 1H), 2.82 (dd, 1H), 3.28−3.31 (m, 1H), 4.19 (dd, 1H), 4.49 (dd, 1H), 5.39 (s, 2H), 6.62 (dd, 1H), 7.20 (d, 1H), 7.34−7.49 (m, 6H), 7.98 (dd, 1H), 8.44 (m, 1H). MS (EI): *m*/*z* (%) 307 (100) [M$^+$], 200 (75).

Benzyl 1-[3-(Biphenyl-4-yloxy)-2-hydroxypropyl]indole-5-carboxylate (10c). Compound **10c** was prepared by coupling **10b** (0.68 g, 2.2 mmol) with 4-phenylphenol (0.37 g, 2.2 mmol) by applying the procedure described for the synthesis of **25c**. The crude product was purified by silica gel chromatography (CH_2Cl_2/ethyl acetate, 95:5) and recrystallized from petroleum ether/ethyl acetate to give **10c** as a solid (0.76 g, 72%); mp 119−123 °C. ^{1}H NMR ($CDCl_3$): δ 2.36 (s, broad, 1H), 3.89−3.93 (m, 1H), 3.99−4.03 (m, 1H), 4.35−4.49 (m, 3H), 5.39 (s, 2H), 6.63 (d, 1H), 6.96 (dd, 2H), 7.24 (d, 1H), 7.30−7.56 (m, 13H), 7.96 (dd, 1H), 8.45 (d, 1H). MS (EI): *m*/*z* (%) 477 (100) [M$^+$], 264 (31).

Benzyl 1-[3-(Biphenyl-4-yloxy)-2-oxopropyl]indole-5-carboxylate (10d). Compound **10c** (0.60 g, 1.3 mmol) was oxidized using the procedure described for the preparation of **25e**. Recrystallization from petroleum ether/ethyl acetate afforded **10d** as a solid (0.24 g, 39%); 126−128 °C. ^{1}H NMR ($CDCl_3$): δ 4.67 (s, 2H), 5.24 (s, 2H), 5.35 (s, 2H), 6.70 (dd, 1H), 6.97−6.99 (m, 2H), 7.11 (d, 1H), 7.14 (d, 1H), 7.32−7.48 (m, 8H), 7.55−7.58 (m, 4H), 7.95 (dd, 1H), 8.45 (d, 1H). MS (EI): *m*/*z* (%) 475 (100) [M$^+$], 264 (42).

1-[3-(Biphenyl-4-yloxy)-2-oxopropyl]indole-5-carboxylic Acid (10). The benzyl ester group of **10d** (0.20 g, 0.42 mmol) was cleaved according to the procedure described for the preparation of **25**. The crude product was purified by silica gel chromatography (petroleum ether/ethyl acetate/acetic acid, 88:10:2) followed by recrystallization from petroleum ether/ethyl acetate to yield **10** as a solid (40 mg, 25%); mp 203−206 °C. ^{1}H NMR (DMSO-d_6): δ 5.11 (s, 2H), 5.47 (s, 2H), 6.64 (d, 1H), 7.07−7.09 (m, 2H), 7.29−7.33 (m, 1H), 7.40−7.50 (m, 4H), 7.61−7.63 (m, 4H), 7.74 (dd, 1H), 8.24 (d, 1H), 12.47 (s, broad, 1H). MS (ESI−): *m*/*z* 384 [M − H]$^-$. MS (ESI+): *m*/*z* 386 [M + H]$^+$.

Allyl Indole-5-carboxylate (16b). A mixture of indole-5-carboxylic acid (**16a**) (1.5 g, 9.3 mmol), allyl bromide (22 mL), $NaHCO_3$ (5.2 g), and DMF (43 mL) was stirred at room temperature for 24 h. After addition of water, the reaction mixture was extracted with ethyl acetate. The organic layer was dried (Na_2SO_4), and the solvent was distilled off. The residue was purified by silica gel chromatography (petroleum ether/ethyl acetate, (a) 9:1; (b) 8:2) to yield **16b** as a solid (1.7 g, 91%); mp 69−70 °C. ^{1}H NMR ($CDCl_3$): δ 4.78 (dt, 2H), 5.21 (dd, 1H), 5.36 (dd, 1H), 5.96−6.05 (m, 1H), 6.58 (d, 1H), 7.19 (d, 1H), 7.33 (d, 1H), 7.86 (dd, 1H), 8.38 (d, 1H). MS (EI): *m*/*z* (%) 201 (25) [M$^+$], 144 (100).

Allyl 1-Oxiranylmethylindole-5-carboxylate (16c). Compound **16c** was synthesized from **16b** (1.7 g, 8.5 mmol) according to the procedure described for the preparation of **25b**. Yield: 1.9 g (87%); mp 68−69 °C. ^{1}H NMR ($CDCl_3$): δ 2.45 (dd, 1H), 2.82 (m, 1H), 3.29−3.31 (m, 1H), 4.19 (dd, 1H), 4.50 (dd, 1H), 4.85 (dt, 2H), 5.29 (dd, 1H), 5.44 (dd, 1H), 6.03−6.13 (m, 1H), 6.62 (d, 1H), 7.16 (d, 1H), 7.38 (d, 1H), 7.93 (dd, 1H), 8.39 (d, 1H). MS (EI): *m*/*z* (%) 257 (45) [M$^+$], 200 (100).

Allyl 1-[3-(3′-Chlorobiphenyl-4-yloxy)-2-hydroxypropyl]indole-5-carboxylate (16d). Compound **16d** was prepared by coupling **16c** (0.26 g, 1.0 mmol) with 4-(3-chlorophenyl)phenol (0.22 g,

1.1 mmol) by applying the procedure described for the synthesis of **25c**. The crude product was purified by silica gel chromatography (petroleum ether/ethyl acetate, 8:2) to give **16d** as a solid (0.31 g, 67%); mp 95−96 °C. ^{1}H NMR ($CDCl_3$): δ 3.90−4.03 (m, 2H), 4.35−4.49 (m, 3H), 4.84 (m, 2H), 5.27 (dd, 1H), 5.43 (dd, 1H), 6.02−6.11 (m, 1H), 6.63 (d, 1H), 6.94−6.97 (m, 2H), 7.23−7.53 (m, 8H), 7.93 (d, 1H), 8.43 (s, 1H). MS (EI): *m*/*z* (%) 461 (58) [M^+], 214 (100).

Allyl 1-[3-(3′-Chlorobiphenyl-4-yloxy)-2-oxopropyl]indole-5-carboxylate (16e). A solution of **16d** (0.30 g, 0.66 mmol) in dry CH_2Cl_2 (8 mL) was treated with Dess−Martin periodinane reagent (0.42 g, 0.99 mmol) and stirred under a nitrogen atmosphere at room temperature for 2 h. Diethyl ether and a solution of sodium thiosulfate (2.1 g) in saturated sodium bicarbonate solution (16 mL) was added. After stirring for 5 min, the reaction mixture was extracted exhaustively with CH_2Cl_2. The combined organic layers were dried (Na_2SO_4) and the solvent was evaporated. The residue was purified by silica gel chromatography (petroleum ether/ethyl acetate, 8:2) to give **16e** as a solid (0.18 g, 59%); mp 123−124 °C. ^{1}H NMR ($CDCl_3$): δ 4.69 (s, 2H), 4.67 (dt, 2H), 5.25 (s, 2H), 5.29 (dd, 1H), 5.43 (dd, 1H), 6.02−6.12 (m, 1H), 6.71 (d, 1H), 6.97−6.99 (m, 2H), 7.12−7.56 (m, 8H), 7.94 (dd, 1H), 8.45 (d, 1H). MS (EI): *m*/*z* (%) 459 (66) [M^+], 214 (100).

1-[3-(3′-Chlorobiphenyl-4-yloxy)-2-oxopropyl]indole-5-carboxylic Acid (16). A solution of **16e** (0.17 g, 0.38 mmol) in dry THF (6 mL) was treated with tetrakis(triphenylphosphin)palladium (0) (0.05 g, 0.04 mmol). After nitrogen was bubbled through the solution for 10 min, acetic acid (0.4 mL) was added and the mixture was stirred at room temperature under a nitrogen atmosphere for 1 h. Then 1.7 g silica gel was added and the solvent was evaporated. The residue was transferred to the top of a silica gel column and elution was carried out with a mixture of petroleum ether/ethyl acetate/acetic acid (a) 9:1:0.1; b) 3:7:0.1) to afford **16** as a solid (90 mg, 56%); mp 228−229 °C. ^{1}H NMR (DMSO-d_6): δ 5.12 (s, 2H), 5.47 (s, 2H), 6.63 (d, 1H), 7.09 (d, 2H), 7.36−7.68 (m, 8H), 7.73 (dd, 1H), 8.24 (d, 1H), 12.45 (s, broad, 1H). MS (ESI+): *m*/*z* 420 [$M + H]^+$.

Methyl 1-Oxiranylmethylindole-5-carboxylate (25b). A mixture of powdered KOH (88%; 0.83 g, 13 mmol), methyl indole-5-carboxylate (**25a**) (1.1 g, 6.3 mmol), tetrabutylammonium bromide (0.20 g, 0.63 mmol), and epichlorohydrin (3 mL) was stirred under a nitrogen atmosphere at room temperature for 1 h and then directly subjected to chromatography on silica gel ((1) petroleum ether/ethyl acetate, 9:1; (2) petroleum ether/ethyl acetate, 8:2) to give **25b** as a solid (0.88 g, 60%); mp 50−51 °C. ^{1}H NMR ($CDCl_3$): δ 2.46 (dd, 1H), 2.81−2.84 (m, 1H), 3.29−3.31 (m, 1H), 3.94 (s, 3H), 4.18 (dd, 1H), 4.49 (dd, 1H), 6.62 (d, 1H), 7.16 (d, 1H), 7.38 (d, 1H), 7.93 (dd, 1H), 8.39 (d, 1H). MS (EI): *m*/*z* (%) 231 (100) [M^+], 188 (72).

Methyl 1-[2-Hydroxy-3-(4-octyloxyphenoxy)propyl]indole-5-carboxylate (25c). Under a nitrogen atmosphere a mixture of **25b** (0.35 g, 1.5 mmol), 4-octyloxyphenol (0.33 g, 1.5 mmol) and 4-dimethylaminopyridine (20 mg, 0.16 mmol) was stirred at 90 °C for 3 h. The reaction mixture was purified by silica gel chromatography (petroleum ether/ethyl acetate, 8:2) followed by recrystallization from petroleum ether/ethyl acetate to afford **25c** as solid (0.60 g, 88%); mp 96−97 °C. ^{1}H NMR ($CDCl_3$): δ 0.85−0.93 (m, 3H), 1.26−1.47 (m, 10H), 1.72−1.79 (m, 2H), 2.39−2.41 (m, 1H), 3.81−3.83 (m, 1H), 3.86−3.99 (m, 6H), 4.34−4.50 (m, 3H), 6.61 (d, 1H), 6.80−6.84 (m, 4H), 7.21 (d, 1H), 7.39 (d, 1H), 7.89 (dd, 1H), 8.40 (d, 1H). MS (EI): *m*/*z* (%) 453 (86) [M^+], 91 (100).

Benzyl 1-[2-Hydroxy-3-(4-octyloxyphenoxy)propyl]indole-5-carboxylate (25d). A mixture of dry benzyl alcohol (11 mL) and KH (30% dispersion in mineral oil, 0.11 g, 0.82 mmol) was stirred at room temperature for 15 min. Then **25c** (0.58 g, 1.3 mmol) was added and the mixture was heated at 100 °C for 2 h, cooled, treated with brine and extracted twice with diethyl ether. The combined organic phases were dried (Na_2SO_4) and concentrated. The residue was dissolved in a small amount of methanol. On adding twice the volume of water followed by cooling in an ice bath with stirring, the product precipitated. The crystals were filtered off by suction, washed with cold methanol/water (1:1), and dried to yield **25d** (0.21 g, 31%); mp 96−97 °C. ^{1}H NMR ($CDCl_3$): δ 0.87 (t, 3H), 1.24−1.46 (m, 10H), 1.72−1.79 (m, 2H), 2.41 (s, broad, 1H), 3.79−3.82 (m, 1H), 3.88−3.92 (m, 3H), 4.32−4.36 (m, 2H), 4.41−4.47 (m, 1H), 5.39 (s, 2H), 6.61 (d, 1H), 6.79−6.83 (m, 4H), 7.21 (d, 1H), 7.32−7.49 (m, 6H), 7.94 (dd, 1H), 8.43 (d, 1H). MS (EI): *m*/*z* (%) 529 (100) [M^+], 327 (72).

Benzyl 1-[3-(4-Octyloxyphenoxy)-2-oxopropyl]indole-5-carboxylate (25e). Compound **25d** (0.20 g, 0.38 mmol) was added to a mixture of acetic anhydride (1.5 mL) and dry DMSO (11 mL), and the resulting mixture was stirred at room temperature overnight. After addition of aqueous $NaHCO_3$ (5%) and brine, the mixture was extracted exhaustively with diethyl ether. The combined organic phases were washed twice with brine, dried (Na_2SO_4), and the solvent was distilled off. The residue was purified by silica gel chromatography (petroleum ether/ethyl acetate, 8:2) to yield **25e** as a solid (0.18 g, 90%); mp 101−103 °C. ^{1}H NMR ($CDCl_3$): δ 0.87 (t, 3H), 1.26−1.47 (m, 10H), 1.73−1.80 (m, 2H), 3.92 (t, 2H), 4.60 (s, 2H), 5.22 (s, 2H), 5.39 (s, 2H), 6.68 (d, 1H), 6.83−6.88 (m, 4H), 7.09 (d, 1H), 7.12 (d, 1H), 7.34−7.49 (m, 5H), 7.95 (dd, 1H), 8.45 (d, 1H). MS (EI): *m*/*z* (%) 527 (100) [M^+], 264 (65).

1-[3-(4-Octyloxyphenoxy)-2-oxopropyl]indole-5-carboxylic Acid (25). A mixture of **25e** (0.20 g, 0.38 mmol), palladium (10%) on charcoal (70 mg), and THF (8 mL) was stirred under a balloon filled with H_2 at room temperature until most of the starting compound had disappeared. After addition of THF (5 mL), the mixture was centrifuged and the supernatant filtered through a membrane filter. The solvent was evaporated and the residue recrystallized from petroleum ether/ethyl acetate to give **25** as a solid (30 mg, 18%); mp 130−132 °C. ^{1}H NMR ($CDCl_3$): δ 0.89 (t, 3H), 1.25−1.48 (m, 10H), 1.74−1.81 (m, 2H), 3.93 (t, 2H), 4.63 (s, 2H), 5.25 (s, 2 H), 6.72 (d, 1H), 6.88 (m, 4H), 7.11−7.15 (m, 2H), 7.97 (dd, 1H), 8.50 (d, 1H). MS (ESI−): *m*/*z* 436 [$M - H]^-$.

Methyl 3-Isobutanoylindole-5-carboxylate (36a). To a mixture of a $ZnCl_2$ solution in dry diethyl ether (2.2 M, 2.8 mL) and dry CH_2Cl_2 (20 mL) was slowly added under nitrogen at 0 °C a solution of butyllithium in dry hexane (1.6 M, 3.5 mL). The mixture was allowed to warm up to room temperature, stirred for 1 h at room temperature, treated with a solution of methyl indole-5-carboxylate (1.0 g, 5.7 mmol) in dry CH_2Cl_2 (20 mL), and further stirred for 1 h. The reaction mixture was cooled to 0 °C, isobutanoyl chloride (1.3 mL, 12 mmol) was added and stirring was continued for 1 h at room temperature. Then the mixture was treated with $AlCl_3$ (0.82 g, 6.2 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 **36a** (1.0 g, 72%); mp 202−203 °C. ^{1}H NMR (DMSO-d_6): δ 1.12−1.13 (m, 6H), 3.44−3.47 (sept, 1H), 3.86 (s, 3H), 7.54 (d, 1H), 7.83 (d, 1H), 8.50 (d, 1H), 8.90 (s, 1H), 12.24 (s, broad, 1H). MS (EI): *m*/*z* (%) 245 (12) [M^+], 202 (100).

Methyl 3-Isobutanoyl-1-oxiranylmethylindole-5-carboxylate (36b). Compound **36b** was synthesized from **36a** (1.0 g, 4.1 mmol), powdered KOH (88%; 0.46 g, 7.2 mmol), tetrabutylammonium bromide (0.13 g, 0.41 mmol), and epichlorohydrin (6 mL) according to the procedure described above for the preparation of **25b**. The product was purified by silica gel chromatography (hexane/ethyl acetate, (a) 9:1; (b) 8:2; (c) 1:1) to give **35b** as an oil (1.16 g, 94%). ^{1}H NMR ($CDCl_3$): δ 1.24−1.27 (m, 6H), 2.47−2.49 (m, 1H), 2.87−2.89 (m, 1H), 3.33−3.37 (m, 2H), 3.93 (s, 3H), 4.11−4.19 (m, 1H), 4.60 (dd, 1H), 7.42 (d, 1H), 7.88 (s, 1H), 8.04 (d, 1H), 9.14 (s, 1H). MS (EI): *m*/*z* (%) 301 (12) [M^+], 258 (100).

Methyl 1-[2-Hydroxy-3-(4-phenoxyphenoxy)propyl]-3-isobutanoylindole-5-carboxylate (36c). Under a nitrogen atmosphere a mixture of **36b** (1.09 g, 3.6 mmol), 4-phenoxyphenol (0.67 g,

3.6 mmol) and 4-dimethylaminopyridine (88 mg, 0.72 mmol) was stirred at 120 °C for 30 min. The warm reaction mixture was dissolved in toluene and then directly purified by silica gel chromatography (hexane/ethyl acetate, 8:2) to afford **36c** as solid (1.18 g, 67%); mp 132−133 °C. ^{1}H NMR ($CDCl_3$): δ 1.21−1.27 (m, 6H), 2.65 (s, broad, 1H), 3.26−3.27 (m, 1H), 3.92 (s, 3H), 3.97−3.99 (m, 2H), 4.41−4.50 (m, 3H), 6.86−6.88 (m, 2H), 6.93−6.99 (m, 4H), 7.05−7.07 (m, 1H), 7.29−7.33 (m, 2H), 7.42 (d, 1H), 7.92 (s, 1H), 7.98 (d, 1H), 9.11 (s, 1H). MS (EI): *m*/*z* (%) 487 (24) [M^+], 444 (100).

Methyl 3-Isobutanoyl-1-[2-oxo-3-(4-phenoxyphenoxy)propyl]-indole-5-carboxylate (36d). Compound **36c** (0.60 g, 1.2 mmol) was oxidized using the procedure described for the preparation of **16e**. Purification by silica gel chromatography (hexane/ethyl acetate, (a) 8:2; (b) 7:3; (c) 1:1) gave **36d** as solid (0.51 g, 87%); mp 91−92 °C. ^{1}H NMR ($CDCl_3$): δ 1.25−1.27 (m, 6H), 3.30 (sept, 1H), 3.93 (s, 3H), 4.71 (s, 2H), 5.33 (s, 2H), 6.92−6.99 (m, 4H), 7.03−7.15 (m, 4H), 7.32−7.36 (m, 2H), 7.78 (s, 1H), 8.01 (d, 1H), 9.15 (s, 1H). MS (EI): *m*/*z* (%) 485 (21) [M^+], 442 (100).

Methyl 1-[2,2-Diethoxy-3-(4-phenoxyphenoxy)propyl]-3-isobutanoylindole-5-carboxylate (36e). A solution of **36d** (0.50 g, 1.03 mmol) in dry ethanol (25 mL) was treated dropwise with orthoformic acid triethylester (1.9 mL, 11 mmol). After addition of concentrated H_2SO_4 (3 drops), the mixture was heated under reflux for 2.5 h. The reaction mixture was poured into 5% aqueous sodium bicarbonate solution and extracted exhaustively with ethyl acetate. The combined organic layers were dried (Na_2SO_4) and the solvent was evaporated. The residue was purified by silica gel chromatography (hexane/ethyl acetate, 8:2) to yield **36e** as a solid (0.33 g, 57%); mp 112−113 °C. ^{1}H NMR ($CDCl_3$): δ 1.17−1.19 (m, 6H), 1.24−1.29 (m, 6H), 3.13 (sept, 1H), 3.65−3.75 (m, 6H), 3.90 (s, 3H), 4.52 (s, 2H), 6.76−6.78 (m, 2H), 6.91−6.93 (m, 4H), 7.04−7.07 (m, 1H), 7.29−7.33 (m, 2H), 7.49 (d, 1H), 7.85−7.88 (m, 2H), 9.09 (s, 1H). MS (EI): *m*/*z* (%) 559 (9) [M^+], 301 (100).

3-Isobutanoyl-1-[2-oxo-3-(4-phenoxyphenoxy)propyl]indole-5-carboxylic Acid (36). A solution of **36e** (283 mg, 0.51 mmol) in methanol (25 mL) was treated with a solution of NaOH (1.6 g, 40 mmol) in water (25 mL). The mixture was heated under reflux for 5 h. After addition of 6 M HCl (30 mL) and THF (35 mL), refluxing was continued for 3.5 h. The reaction mixture was concentrated until some precipitates appeared and then extracted exhaustively with ethyl acetate. The combined organic layers were dried (Na_2SO_4) and the solvent was distilled off. Chromatography on silica gel (hexane/ethyl acetate/formic acid, (a) 8:2:0.1; (b) 2:8:0.1) afforded **36** as a solid (153 mg, 64%); mp 103−104 °C. ^{1}H NMR (DMSO-d_6): δ 1.13−1.15 (m, 6H), 3.32−3.39 (m, 1H), 5.07 (s, 2H), 5.54 (s, 2H), 6.91−6.93 (m, 2H), 6.99−7.09 (m, 5H), 7.32−7.36 (m, 2H), 7.57 (d, 1H), 7.82 (d, 1H), 8.39 (s, 1H), 8.88 (s, 1H), 12.66 (s, broad, 1H). ^{13}C NMR (DMSO-d_6): δ 20.33, 36.99, 53.63, 71.96, 111.43, 115.77, 116.67, 118.13, 121.24, 123.42, 124.70−124.74, 125.24−126.20, 130.61, 139.54, 140.58, 150.76, 154.65, 158.64, 168.65, 199.88, 201.15. MS (ESI+): *m*/*z* 472 [M + H]$^+$.

1-[2-Hydroxy-3-(4-phenoxyphenoxy)propyl]-3-isobutanoylindole-5-carboxylic Acid (37). A mixture of **36c** (200 mg, 0.41 mmol), EtOH (15 mL), and 10% aqueous KOH (5 mL) was heated under reflux for 30 min, cooled, acidified with dilute HCl, and extracted with diethyl ether. The organic phase was dried (Na_2SO_4) and the solvent was evaporated. The residue was recrystallized from petroleum ether/ethyl acetate to yield **37** as a solid (43 mg, 22%). mp 148 °C. ^{1}H NMR ($CDCl_3$): δ 1.20−1.25 (m, 6H), 3.22−3.33 (m, 1H), 3.91−3.97 (m, 1H), 3.99−4.05 (m, 1H), 4.37−4.58 (m, 3H), 6.86−6.91 (m, 2H), 6.91−7.01 (m, 4H), 7.03−7.09 (m, 1H), 7.28−7.34 (m, 2H), 7.44 (d, 1H), 7.94 (s, 1H), 8.01 (d, 1H), 9.15 (s, 1H). MS (EI): *m*/*z* (%) 473 (13) [M^+], 430 (100).

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 and bile 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 Millenium32 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.[34] 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 centrifugation of the samples and cleaning up the supernatant by solid phase extraction. Inhibition of cPLA$_2\alpha$ activity was calculated by comparing the arachidonic acid formed by the enzyme in absence and presence of a test compound. In each incubation series, compound **5** was tested as reference at a concentration of 33 nM to confirm the consistency of the inhibition data. Inhibition of cPLA$_2\alpha$ activity produced by **5** at this concentration was 47 ± 6% (mean ± standard deviation, n = 12) in these experiments.

2.2.2. Cellular Assay. The ability of compound **36** 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.[35] Briefly, platelets were isolated from human buffy coat by differential centrifugation and washed with phosphate buffered saline (pH 7.4). After addition of the stimulants 12-*O*-tetradecanoylphorbol-13-acetate (TPA) (2 μM) the cells were incubated for 60 min. Incubation was carried out in presence of the 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 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 the supernatant by solid phase extraction.

2.3. Metabolic Stability. Metabolism of the target compounds was studied as described previously using rat liver microsomes.[35] 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 (see below) found in presence and absence of NADPH.

To elucidate the structure of the respective main metabolites, HPLC-ESI-MS experiments were performed in the way recently reported.[35] In each ESI-mass chromatogram (negative ion mode) besides the expected [M − H]$^-$ ions of the target compounds, hydrate forms of these activated ketones with [M + 18 − H]$^-$ were observed. For the main metabolites, which eluted without exception between the hydrate form and the ketone form of the test compounds, [M + 2 − H]$^-$ ions were detected, which were assigned to the secondary alcohols produced by reduction of the activated ketone groups of the target compounds.

2.4. Solubility. Thermodynamic solubility was determined in the way previously described.[35] 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 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 ketone form 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 applying a published OECD method.[37] The following reference compounds were used: anisole, acetophenone, ethyl benzoate, benzophenone, naphthalene, diphenyl, phenanthrene, dibenzyl, fluoranthene, triphenylamine, and DDT. To 2 μL of a stock solution of a reference and a test compound, respectively, in DMSO (5 mM) was added 2 μL of a solution of thiourea in DMSO (5 mM) applied for dead time determination and 1 mL of the mobile phase, which consisted of acetonitrile/water/phosphoric acid (85%) (55:45:0.1, v/v/v). From each sample, 20 μL were injected onto the HPLC system. Analysis was performed on a Kromasil 100−5C18 column (3 mm I.D. × 60 mm, particle size 5 μm). The temperature of the column oven was maintained at 25 °C. The flow rate was 0.6 mL/min and the effluents were monitored at 254 nm.

The linear fit of the log *k* values (*k*: capacity factor) and the log *P* values of the 11 references showed a correlation factor of $R = 0.9965$. With this method, for indomethacin and ibuprofen, log *P* values of 2.9 and 3.2, respectively, were obtained. For measuring the log *P* values of the target compounds, the retention times of the peaks of their ketone forms were used.

2.6. Bioavailability. 2.6.1. Determination of 36 and Its Metabolite 37 in Mouse Plasma. The pharmacokinetic studies with compound **36** were performed with C57Bl6 mice. For iv administration, animals ($n = 3$) anesthetized through inhalation of isoflurane (2%, 0.5 L/min O_2) received a single dose of 10 mg/kg via tail vein injection (2 μL per g mouse of a solution of 2 mg **36** in 500 μL DMSO/phosphate buffered saline pH 7.4, 2:1, v/v). From each mouse, venous blood samples were collected from the retroorbital vein plexus in heparinized syringes after 5 and 30 min (about 250 μL each). For po administration, animals ($n = 3$) were dosed once via gavage with 100 mg/kg **36** (8 μL per g mouse of a suspension of 12.5 mg **36** in 1000 μL of a 1% aqueous methylcellulose Methocel A4C, 408 mPas, Fa. Colorcon, solution). Again, blood samples were withdrawn from each animal after 1 and 2 h. Control experiments with indomethacin were performed in the same way dosing the mice ($n = 4$) perorally with 10 mg/kg (8 μL per g mouse of a suspension of 2.5 mg indomethacin in 2000 μL of a 1% aqueous methylcellulose solution) and collecting blood after 1 and 2 h. All blood samples were centrifuged at 3000*g* for 20 min, and the obtained plasma (about 100 μL each) was separated.

To 100 μL of each blood plasma were added 395 μL of phosphate buffered saline (pH 7.4), 5 μL of a DMSO solution of the internal standard 4-hexyloxybenzoic acid (1.0 mg/mL), and 500 μL of acetonitrile. After vortexing, 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. Separation was achieved on a Phenomenex Aqua C18 analytical column (4.6 mm I.D. × 75 mm, particle size 3 μm) protected with a Phenomenex C18 security guard column (3 mm I.D. × 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%) (60:40:0.1, v/v/v). The flow rate was 0.7 mL/min and absorption wavelength was set to 235 nm. The amounts of **36**, its alcohol metabolite **37**, and of indomethacin, respectively, were determined using standard curves of the compounds.

2.6.2. Determination of 36 and 37 and Their Glucuronides in the Bile of Mice. In the pharmacokinetic studies with **36**, the animals were killed after the final blood withdrawel (30 min after iv and 2 h after po administration), and bile of each animal was collected.

Direct Measurement of 36 and 37 in Bile. To 2 μL of the bile samples were added 1 μL of a DMSO solution of the internal standard 4-hexyloxybenzoic acid (1.0 mg/mL), 97 μL of phosphate buffered saline (pH 7.4), and 100 μL of acetonitrile. After vortexing, 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. Separation was achieved on a Phenomenex Aqua C18 analytical column (4.6 mm I.D. × 100 mm, particle size 3 μm) protected with a Phenomenex C18 security guard column (3 mm I.D. × 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%) (60:40:0.1, v/v/v). The flow rate was 0.7 mL/min and absorption wavelength was set to 235 nm. The amounts of **36** and its alcohol metabolite **37**, respectively, were determined using standard curves of the compounds.

Measurement of 36 and 37 in Bile after Treatment with Glucuronidase. To 2 μL of the bile samples were added 1 μL of a DMSO solution of the internal standard 4-hexyloxybenzoic acid (1.0 mg/mL), 177 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 Art. 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. The injection volume was 200 μL.

Measurement of the Glucuronides of 36 and 37 in Bile with HPLC/MS. Obtained solutions were also investigated by HPLC/high resolution mass spectrometry. The HPLC system consisted of an Ultimate 3000 RS Dionex system coupled with a Bruker micrOTOF-Q II detector. System control and data proccesing was performed with Hystar and DataAnalysis (Bruker, Germany). The reversed phase chromatography was carried out on a C18 Aqua column (2 mm I.D. × 75 mm, particle size 3 μm) at a flow rate of 0.4 mL/min utilizing the following gradient program. The gradient started from 70% A (acetonitrile/10 mM aqueous ammonium formate/formic acid 10:90:0.1, v/v/v) and 30% B (acetonitrile/10 mM aqueous ammonium formate/formic acid 90:10:0.1, v/v/v). The gradient was run linearly to 35% solvent A within 10 min and then to 0% solvent A within 1 min. After further 3 min, the amount of solvent A was linearly increased again to 70% over 1 min, and this ratio was held for 3 min.

The electrospray ion source was operated in negative ionization mode, scan range of m/z 150−1000. The capillary was set to −3.5 kV, the nebulizer was operated at 0.4 bar, and the dry gas was set to 4 L/min at a temperature of 180 °C. Transfer voltages of funnel 1 and 2 were set to 200 Vpp. The hexapole RF voltage was set to 100 Vpp. Mass calibration was done using 20 mM aqueous lithium formate solution infused at the end of the chromatography.

Results. The samples, which were not treated with glucuronidase, contained high concentrations of glucuronides of **36** and **37**, as confirmed by their high resolution mass peaks of m/z 646.2105 and m/z 648.2216 ($[M - H]^-$). In the samples treated with glucuronidase increased concentrations of **36** and **37** occurred, while their glucuronides could not be detected any more.

2.7. Benzalkoniumchloride-Induced Murine Ear Edema Model. Benzalkonium chloride-induced ear inflammation was elicited according to described methods,[38,39] with some modifications. Test solutions of **36** were prepared by dilution of an aliquot of a stock solution of **36** in acetone (10%, m/v) with acetone to the final concentration. For the induction of irritative contact eczema, 10 μL of 5% benzalkonium chloride (Sigma, Deisenhofen, Germany) dissolved in olive oil/acetone (1:4, v/v) was applied to the dorsal surface of the right ears. After 10 min, 10 μL of the test solutions of **36** (final amounts of **36**: 0.01, 0.1, and 1 mg/ear), or 10 μL of the vehicle (acetone) were applied onto the

benzalkonium chloride treated ears. Left ears received vehicle only and served as controls. Ear swelling was measured over three consecutive days with a venire calliper and compared to the contra lateral control ear. Values represent means of five BALB/c mice per group.

In a second independent experiment, the anti-inflammtory effect of **36** in the acute irritant contact dermatitis model at a dose of 0.1 mg/ear was compared with the effect of the potent glucocorticoid clobetasol-17-propionate (Karison Crinale Solution, 0.5 mg/g, Dermapharm, Germany) at a dose of 0.005 mg/ear. The experiment was performed as described above with three groups of mice treated at different days, each group consisting of five mice (animal experiments were approved by the state review board (Münster, G24/96)).

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Supporting Information Available: Purification procedure, melting points, ^{1}H NMR and MS data of target compounds **8**, **9**, **11**−**15**, **17**−**24**, and **26**−**35**; assay for iPLA$_2$ and sPLA$_2$ inhibition; HRMS-HPLC spectra of **36** and **37** and their glucuronides in the bile of mice treated with **36**. This material is available free of charge via the Internet at http://pubs.acs.org.

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