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1-(2-Carboxyindol-5-yloxy)propan-2-ones as inhibitors of human cytosolic phospholipase $A_2\alpha$: Synthesis, biological activity, metabolic stability, and solubility

Alexandra Fritsche, Alwine Schulze Elfringhoff, Jörg Fabian and Matthias Lehr*

Institute of Pharmaceutical and Medicinal Chemistry, University of Münster, Hittorfstrasse 58-62, D-48149 Münster, Germany

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Abstract—Indole-5-carboxylic acids with 3-aryloxy-2-oxopropyl residues in position 1 were previously reported to be potent inhibitors of cytosolic phospholipase $A_2\alpha$ ($cPLA_2\alpha$) isolated from human platelets. In continuation of our attempts to develop novel $cPLA_2\alpha$ inhibitors, a series of structurally related indole-2-carboxylic acids containing 3-aryloxy-2-oxopropoxy residues in position 5 were synthesized and tested for their $cPLA_2\alpha$-inhibitory potency. Furthermore, the thermodynamic solubility of these compounds and their metabolic stability against rat liver microsomes were evaluated.
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1. Introduction

Cytosolic phospholipase $A_2\alpha$ ($cPLA_2\alpha$) is an esterase that selectively cleaves the *sn*-2 position of arachidonoyl-glycerophospholipids of biomembranes to generate free arachidonic acid and lysophospholipids.[1] Arachidonic acid in turn is metabolized to a variety of inflammatory mediators including prostaglandins and leukotrienes. When the phospholipid substrate of $cPLA_2\alpha$ is a phosphatidylcholine with an alkyl ether moiety at the *sn*-1 position, the lysophospoholipid produced is the immediate precursor of platelet activating factor (PAF), another mediator of inflammation.[2] Thus, the inhibition of $cPLA_2\alpha$ would lead to the blockade of the cellular production of these proinflammatory lipid mediators. Therefore, this enzyme is considered to be an attractive target for the design of new anti-inflammatory drugs.[3–5]

First-generation $cPLA_2\alpha$ inhibitors were analogues of arachidonic acid with the COOH group replaced by $COCF_3$ ($AACOCF_3$, **1**) (Fig. 1) or $CH_2PO(OCH_3)F$ (MAFP).[3] Besides other agents, several 1,3-diaryloxypropan-2-ones, such as compound **2**, have been found to be very potent inhibitors of $cPLA_2\alpha$ later on.[6] An important part of the pharmacophore of these substances is the activated electrophilic ketone moiety, which is supposed to form covalent-binding interactions with a serine of the active site of $cPLA_2\alpha$. Recently, we have published effective $cPLA_2\alpha$ inhibitors like compound **3**, structurally related to **2**.[7,8] In these derivatives, a 5-carboxyindol-1-yl-substituent is tethered to an aryloxypropan-2-one scaffold.

Here we describe the synthesis of novel $cPLA_2\alpha$ inhibitors with electrophilic activated ketone groups such as **11**, in which position 1 of 3-aryloxypropan-2-one is bound via an oxygen to the 5 position of indole-2-carboxylic acid. Because it is known that activated ketones can be unstable towards keto-reduction,[9–11] and because reduction of the keto groups of **2** and **3** to alcohol functionalities leads to inactive compounds,[6,11,12] we also tested the metabolic stability of all newly synthesized target compounds in a test system applying rat liver microsomes. Furthermore, we also measured the solubility of the test compounds, in view of the fact that solubility is one important constraint for oral bioavailability.

2. Chemistry

Indole-2-carboxylic acid derivative **11** was synthesized by the route outlined in Scheme 1. 5-Benzyloxyindole-

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* Corresponding author. Tel.: +49 251 83 33331; fax: +49 251 83 32144; e-mail: lehrm@uni-muenster.de

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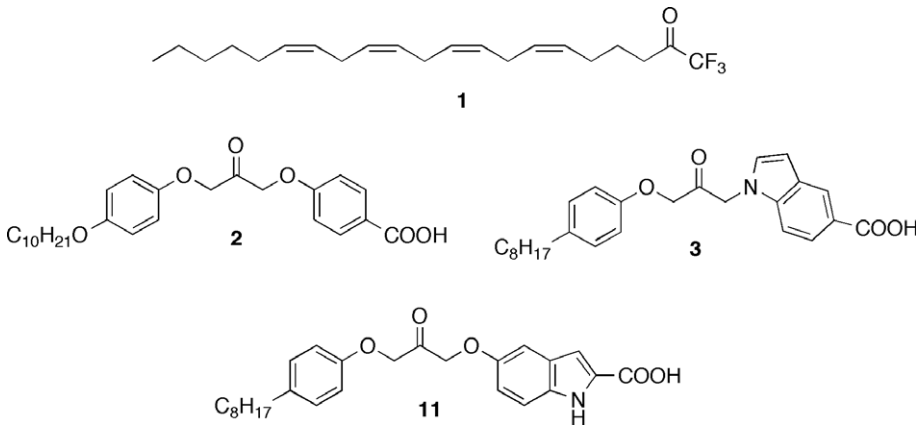
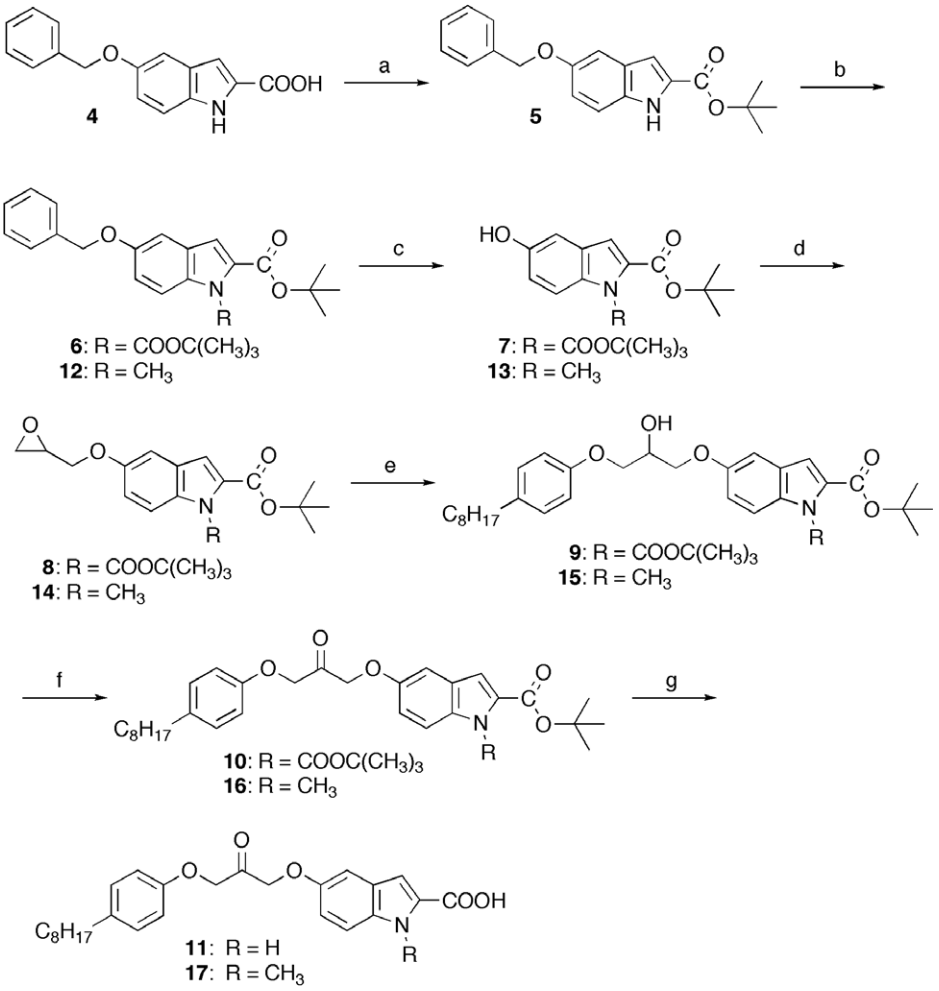


Figure 1.



Scheme 1. Reagents and conditions: (a) *N*,*N*-Dimethylformamide di-*tert*-butyl acetal, benzene, reflux; (b) di-*tert*-butyl dicarbonate, 4-dimethylaminopyridine, THF, rt; (c) H_2, Pd/C, triethylamine, THF, rt; (d) epichlorohydrin, tetrabutylammonium bromide, $KHCO_3$ or KOH, acetonitrile, rt; (e) 4-octylphenol, 4-dimethylaminopyridine, 40 °C or 80–100 °C; (f) acetic anhydride, DMSO, rt; (g) trifluoroacetic acid, CH_2Cl_2, rt.

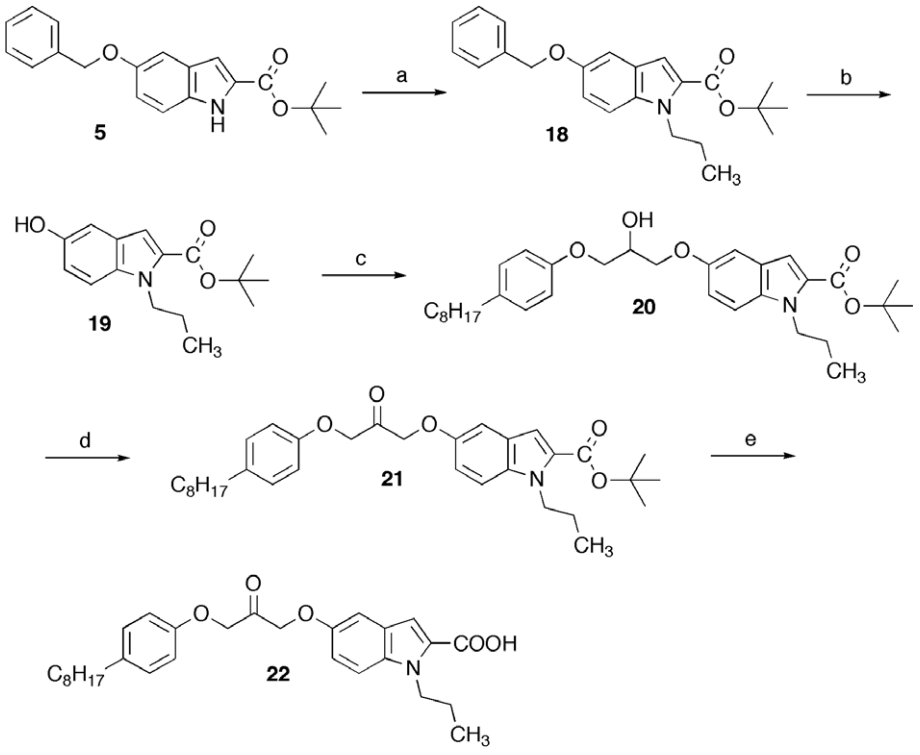
2-carboxylic acid (**4**) was converted to its *tert*-butylester (**5**) by *N*,*N*-dimethylformamide di-*tert*-butyl acetal.[13] Treatment of **5** with di-*tert*-butyl dicarbonate in the presence of 4-dimethylaminopyridine led to the indole-1,2-dicarboxylic acid di-*tert*-butylester **6**. Hydrogenolytic cleavage of the benzyl ether of **6** yielded the phenol **7**. This compound was further reacted with epichlorohydrin in the presence of tetrabutylammonium bromide and $KHCO_3$ to afford the epoxide intermediate **8**. Ring opening of **8** with 4-octylphenol was achieved without solvent in the presence of catalytic amounts of 4-dimethylaminopyridine. Oxidation of the resulting alcohol **9** to

ketone **10** was carried out with acetic anhydride/DMSO. Concomitant deprotection of the indole nitrogen and cleavage of indole-2-carboxylic acid *tert*-butyl ester by trifluoroacetic anhydride yielded target compound **11**.

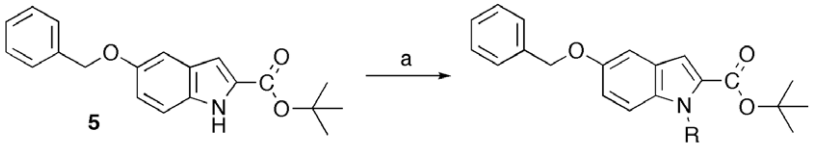
The synthesis of the *N*-methylated indole-2-carboxylic acid **17** is also shown in Scheme 1. 5-Benzyloxyindole-2-carboxylic acid *tert*-butylester (**5**) was methylated in position 1 with methyl *p*-toluenesulfonate to give **12**. After deprotection by hydrogenolysis, the phenolic hydroxy group was reacted with epichlorohydrin applying KOH as base. Ring opening of the epoxide, oxidation of the resulting hydroxy group and final cleavage of the *tert*-butyl ester were performed in the same way as described for the synthesis of **11**.

The preparation of *N*-propyl indole derivative **22** also started from **5** (Scheme 2). Alkylation of this compound with 1-bromopropane in the presence of *tert*-BuOK in DMSO provided **18**. After hydrogenolytic debenzylation, solvent-free reaction of intermediate **19** with 2-(4-octylphenoxy)methyloxirane in the presence of 4-dimethylaminopyridine led to the hydroxy compound **20**. Subsequent oxidation of the alcohol with acetic anhydride/DMSO and cleavage of the *tert*-butyl ester by trifluoroacetic acid gave the desired acid **22**.

The 1-functionalized target compounds **31**–**36** were prepared starting from the appropriately substituted 5-benzyloxyindolecarboxylates **23**–**29** (Scheme 3). The latter compounds were obtained by reaction of **5** with 1-bromohexane, benzylbromide, ω-bromoalkanoic acid *tert*-butylesters, 4-bromomethylbenzoic acid *tert*-butylester and 2-chloro-*N*,*N*-dimethylethylamine, respectively. For synthesis of **30** and **31**, the route used for the preparation of **22** was applied. Compounds **32**–**36** were

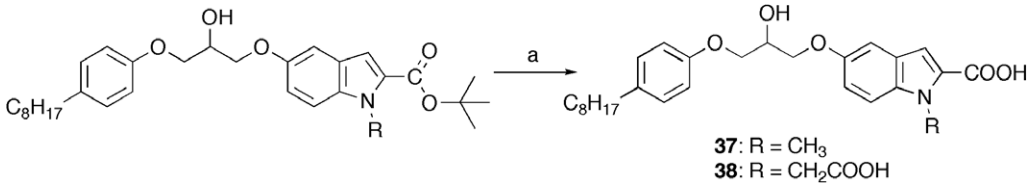


Scheme 2. Reagents and conditions: (a) 1-Bromopropane, *tert*-BuOK, DMSO, 110 °C; (b) H_2, Pd/C, triethylamine, THF, rt; (c) 2-(4-octylphenoxymethyl)oxirane, 4-dimethylaminopyridine, 80–100 °C; (d) acetic anhydride, DMSO, rt; (e) trifluoroacetic acid, CH_2Cl_2, rt.



23: R = C_6H_{13}
24: R = benzyl,
25: R = -$CH_2COOC(CH_3)_3$
26: R = -$(CH_2)_3COOC(CH_3)_3$
27: R = -$(CH_2)_5COOC(CH_3)_3$
28: R = -CH_2phenyl(4-$COOC(CH_3)_3$)
29: R = -$CH_2CH_2N(CH_3)_3$

Scheme 3. Reagent and condition: (a) R–Br or R–Cl, *tert*-BuOK, DMSO, 110 °C.



Scheme 4. Reagent and condition: (a) Trifluoroacetic acid, CH_2Cl_2, rt.

obtained in the manner as described above for the synthesis of **17**.

The indole-2-carboxylic acids **37** and **38** bearing hydroxy groups in the side chain at position 5 of the indole (Scheme 4) were prepared by cleavage of the corresponding *tert*-butyl esters with trifluoroacetic acid.

3. Evaluation of the target compounds

3.1. Inhibition of cPLA$_2\alpha$

All newly synthesized indole derivatives were evaluated in an assay applying cPLA$_2\alpha$ isolated from human platelets.[12] Like other lipases, cPLA$_2\alpha$ has evolved to work optimally at a lipid-water interface. For this reason, sonicated covesicles consisting of 1-stearoyl-2-arachidonoyl-*sn*-glycero-3-phosphocholine and 1,2-dioleoyl-*sn*-glycerol were used as enzyme substrate. A possible problem of assays using such an aggregated form of phospholipids is that a test compound could inhibit the enzyme not by binding to its active site but merely by altering the substrate assembly and hence causing the enzyme to desorb from the lipid-water interface. To exclude this path of action, the mole fraction of inhibitor in the interface has to be kept low.[14] Thus, the highest concentration of test compounds evaluated was 10 μM, whereas the concentration of the vesicle-forming lipids was 300 μM. The enzyme activity was determined by measuring the enzyme product arachidonic acid formed after an incubation time of 60 min with reversed phase HPLC and UV-detection at 200 nm.

Several compounds were also tested in cellular situation. In this assay cPLA$_2\alpha$ of intact human platelets was activated with the phorbol ester 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA).[7] The cPLA$_2\alpha$-catalyzed liberation of arachidonic acid from membrane phospholipids was measured after 60 min with HPLC and UV-detection at 200 nm. To avoid the 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.

3.2. Metabolic stability

Test compounds were incubated with rat liver microsomes under aerobic conditions in the absence and presence of the cofactor NADPH in a similar manner as described previously.[11,15] The metabolic reactions were terminated by addition of acetonitrile after 30 min. Then the mixtures were cooled and centrifuged. Aliquots of the supernatants were subjected to reversed phase HPLC/UV for determination of the extent of metabolism. The structures of the main metabolites were confirmed with reference substances and LC/MS investigations, respectively.

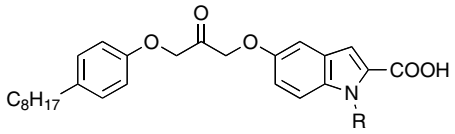
3.3. Water solubility

Water solubility was determined experimentally according to a published procedure.[16] Sodium phosphate buffer (pH 7.4) was added to a test compound, and the suspension obtained was equilibrated by sonication and shaking at room temperature, 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/UV using a regression curve.

4. Results and discussion

Our investigations started from the *N*-unsubstituted indole-2-carboxylic acid **11**. With an IC_{50} of 3.3 μM this compound inhibited isolated cPLA$_2\alpha$ with similar potency as the known cPLA$_2\alpha$ inhibitor AACOCF$_3$ (**1**) (Table 1). In comparison with the structurally related indolylpropan-2-one **3**, however, **11** was about 100-fold less active. Introduction of small lipophilic substituents at the indole 1 position slightly increased activity, whereas bigger lipophilic groups were not tolerated well by the enzyme. Thus, IC_{50}-values evaluated for the methyl (**17**) and propyl (**22**) derivatives were about 1–2 μM and for the hexyl (**30**) and benzyl (**31**) compounds about 10 μM. One reason for the reduced activity of **30** and **31** could be that their terminal lipophilic hydrocarbon groups come close to a hydrophilic domain of the enzyme resulting in negative interactions. To obtain evidence for this possibility and to increase polarity and solubility of the compounds, several ω-carboxyalkyl-chains and a 4-carboxybenzyl-substituent, respectively, were introduced at the indole nitrogen. With IC_{50}-values of 0.3–0.6 μM the carboxymethyl, 3-carboxypropyl- and 5-carboxypentyl-substituted indoles **32**–**34** showed significantly higher cPLA$_2\alpha$ inhibition than the starting compound **11**, probably because of additional interactions of the new polar moieties with the enzyme. The derivative with a more rigid 4-carboxybenzyl-substituent (**35**) (IC_{50}: 1.5 μM) was about threefold less active than the ω-carboxyalkyl-derivatives **32**–**34**. Introduction of a basic dimethylaminoethyl-moiety at position 1 (**36**) even led to a compound with an IC_{50} greater than 10 μM.

Table 1. cPLA$_2\alpha$-inhibitory potency, thermodynamic solubility and metabolic stability of indole-2-carboxylic acid derivatives



Compound	R	Inhibition of cPLA$_2\alpha$ IC$_{50}$[a] (μM)	Metabolic stability[b] (%)	Thermodynamic solubility[c] (μg/mL)
11	H	3.3	<5	8.4 ± 2.0
17	CH_3	1.2	<5	<1
22	C_3H_7	2.1	<5	<1
30	C_6H_{13}	> 10[d]	<5	<1
31	Benzyl	10	<5	<1
32	CH_2COOH	0.3	18	8.1 ± 2.7
33	$(CH_2)_3COOH$	0.4	<5	30 ± 2.5
34	$(CH_2)_5COOH$	0.6	<5	17 ± 0.6
35	CH_2phenyl(4-COOH)	1.5	15	13 ± 3.2
36	$(CH_2)_2N(CH_3)_2$	>10[e]	9	46 ± 9.1
1 (AACOCF$_3$)		2.3	n.t.[f]	n.t.[f]
3		0.035	65 ± 5	<1

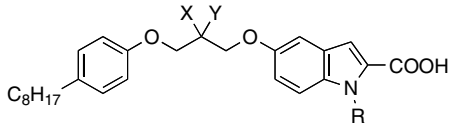
[a] Values are means of at least two independent determinations, errors are within ±20%.
[b] Percentage of parent compound remaining after metabolism by rat liver microsomes; values are the mean of at least two independent determinations; in case of **3**: means ± standard deviation, $n = 5$.
[c] Mean ± standard deviation, $n = 3$, in case of values <1 μg/mL: $n = 2$.
[d] 42% inhibition at 10 μM.
[e] 32% inhibition at 10 μM.
[f] n.t., not tested.

Structure–activity relationship studies have revealed that important pharmacophoric elements of the cPLA$_2\alpha$ inhibitors **2** and **3** are their electrophilic ketone moieties. Reduction of the ketones to the corresponding alcohols led to a drastic loss of activity.[6,7,11] The same effect was observed for the novel inhibitor class. Although the indoles **17** and **32**, which were examined as representatives, showed IC$_{50}$ values against cPLA$_2\alpha$ of 1.2 μM and 0.3 μM, respectively, their alcohol derivatives **37** and **38** were inactive at the highest test concentration of 10 μM (Table 2).

The most active ketones (**17**, **32**–**34**) and the inactive alcohols **37** and **38** were also tested in cellular situation. In intact human platelets, cPLA$_2\alpha$ activity triggered by the phorbol ester TPA was inhibited by **17** and **32**–**34** to about the same extent as the activity of the isolated enzyme (IC$_{50}$ range 0.3–1.3 μM) (Table 2). As it could be expected, the alcohols **37** and **38** were lacking activity at 10 μM also in the cellular assay.

Because it is known that activated ketones may be metabolically unstable,[9–11] the metabolism of the new compounds by rat liver microsomes was also investigated. Figure 2 shows typical chromatograms obtained after incubation of the substances (here compound **32**) with microsomes in the absence and presence of NADPH. Metabolism of the new inhibitors and reference compound **3** was not measured after ether extraction with normal phase HPLC as published previously for **3**, but

Table 2. Inhibition of cPLA$_2\alpha$ activity in the isolated enzyme assay and in the cellular assay



Compound	R	X,Y	Vesicle assay with the isolated enzyme IC$_{50}$[a] (μM)	Cellular assay with intact human platelets (stimulant TPA) IC$_{50}$[a] (μM)
17	CH_3	=O	1.2	0.6
32	CH_2COOH	=O	0.3	1.3
33	$(CH_2)_3COOH$	=O	0.4	0.3
34	$(CH_2)_5COOH$	=O	0.6	0.5
37	CH_3	H, OH	n.a.[b]	n.a.[b]
38	CH_2COOH	H, OH	n.a.[b]	n.a.[b]
3			0.035	0.018

[a] Values are the means of at least two independent determinations; errors are within ±20%.
[b] n.a., not active at 10 μM.

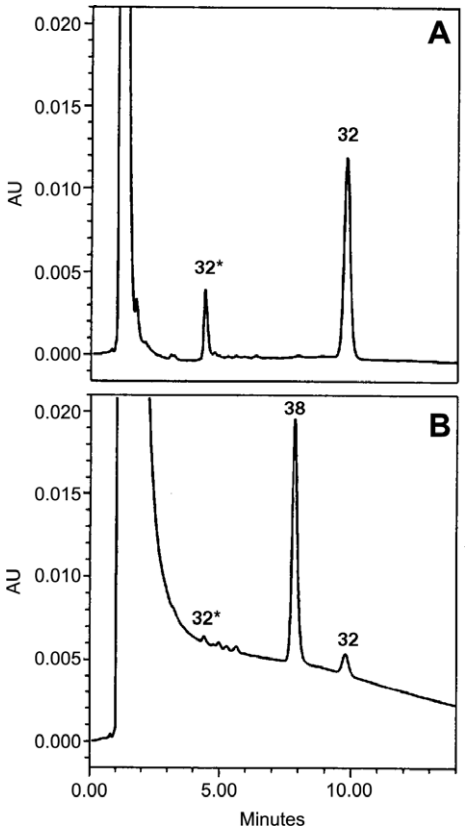


Figure 2. Reversed phase HPLC chromatograms of **32** following incubation with rat liver microsomes in the absence (A) and in the presence (B) of NADPH. **32***: hydrate form of **32**; **38**: corresponding alcohol of **32** (main metabolite). Column: Phenomenex Aqua 3 μ C_{18} column (4.6 mm inside diameter × 75 mm); solvent: acetonitrile/water/phosphoric acid (85%) (70:30:0.1, v/v/v); flow: 0.7 ml/min; UV-detection: 240 nm.

directly by reversed phase HPLC after protein precipitation with acetonitrile. Chromatography under aqueous conditions led to the formation of second peaks, which were identified as the hydrate forms of the electrophilic ketones by LC–MS.[11] However, since the ketone peaks of the compounds in the chromatograms increased linearly with the concentration of the substances in the concentration range evaluated, the metabolism could be measured by comparing only the ketone peaks formed in the presence and absence of NADPH. The ratio of metabolism of reference compound **3** evaluated with this reversed phase method (65%) was nearly identical with the value obtained with the normal phase HPLC system.[11] All new substances were significantly less stable against keto reduction than **3**. After incubation with rat liver microsomes in the presence of NADPH, only about 10–20% of **32**, **35** and **36** and less than 5% of the other compounds of this series could be detected in comparison with reference incubations in the absence of NADPH (Table 1). In all cases the corresponding inactive alcohols were the main metabolites as shown by LC–MS experiments.

Due to the two long acyl chains, the phospholipid substrates of $cPLA_2\alpha$ possess a substantial lipophilicity. Therefore it can be expected that inhibitors, which shall bind competitively to the active site of the enzyme, must have similar properties. This assumption is confirmed by the fact that all known $cPLA_2\alpha$ inhibitors with pronounced potency bear larger lipophilic residues,[6,7,17–21] which lead 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 solubility of all new compounds in phosphate buffer (pH 7.4) under thermodynamic conditions. The amount of test compound dissolved was determined by reversed phase HPLC. Like in the metabolism experiments (Fig. 2A), the ketone peaks of the chromatograms were used for quantification.

The new 1-unsubtituted indole-2-carboxylic acid **11** possesses a significant higher water solubility than reference compound **3** (8.4 μg/mL vs. <1 μg/mL) (Table 1). Introduction of lipophilic methyl, propyl, hexyl or benzyl substituents at the indole-1-position (**17**, **22**, **30**, **31**) decreased the amount of dissolvable substance to less than 1 μg/mL. In contrast, with a value of 8.1 μg/mL the compound with carboxymethyl residue at position 1 (**32**) showed similar solubility as the unsubstituted derivative **11**. Replacement of carboxymethyl by the larger carboxylic acid residues carboxypropyl (**33**), carboxylpentyl (**34**) and 4-carboxybenzyl (**35**) further increased experimental solubility about 1.5- to 4-fold. Introduction of a basic dimethylaminoethyl moiety at indole-1-position finally led to a compound (**36**), which was even more soluble (46 μg/mL) than the best soluble dicarboxylic acid of the synthesized series (**33**, 30 μg/mL).

Solubility guidelines for drugs under development are given by Lipinski and coworkers.[22] According to those, compounds with mid-range permeability and average potency should possess a minimum thermodynamic solubility of 50 μg/mL. With solubilities of 30 μg/mL and 46 μg/mL, respectively, compounds **33** and **36** come closest to this limit.

In conclusion, we have described a new series of $cPLA_2\alpha$ inhibitors with activated ketone groups. Although the inhibitory activity against $cPLA_2\alpha$ and the stability against metabolic keto reduction of these compounds were lower than that of the structurally related reference compound **3** published by us recently, several of the new compounds possessed a significantly increased aqueous solubility in comparison with **3**. The aim of further studies will be to develop derivatives of these compounds, concomitantly possessing high $cPLA_2\alpha$-inhibitory potency, adequate water solubility and good stability against metabolic reduction of the pharmacophoric ketone group.

5. Experimental

5.1. Chemistry

5.1.1. 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 appa-

ratus and are uncorrected. ^{1}H NMR spectra were recorded on a Varian Mercury Plus 400 spectrometer (400 MHz). Mass spectra were obtained on Finnigan GCQ and LCQ apparatuses applying electron beam ionization (EI). With the exception of **36**, 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 (ID) × 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 (ID) × 250 mm, Merck, Darmstadt, Germany) with an isohexane/THF gradient containing 0.1% trifluoroacetic acid at a flow rate of 0.5 mL/min. All these compounds showed purities greater than 95% in both HPLC systems. The purity of **36** was 97%, evaluated on a reversed phase (Symmetry C18, 3.5 μm, 4.6 mm (ID) × 75 mm, Waters, Eschborn, Germany) at 254 nm applying acetonitrile/water with 0.1% phosphoric acid (85%) as mobile phase at a flow rate of 0.7 mL/min.

5.1.2. *tert*-Butyl 5-benzyloxyindole-2-carboxylate (5). To a boiling suspension of 5-benzyloxyindole-5-carboxylic acid (5.0 g, 18.7 mmol) and dry benzene (100 mL) was added dropwise under a nitrogen atmosphere over a period of 45 min a solution of *N*,*N*-dimethylformamide di-*tert*-butyl acetal (15.2 g, 74.9 mmol) in dry benzene (50 mL). After being refluxed for further 2 h, the solvent was removed under reduced pressure and the residue was purified by chromatography on silica gel (hexane/ethyl acetate, 20:1) to afford **5** as solid (3.15 g, 52%); mp 124 °C. ^{1}H NMR ($CDCl_3$): δ 1.61 (s, 9H), 5.10 (s, 2H), 7.04–7.08 (m, 2H), 7.15 (d, 1H), 7.30–7.35 (m, 2H), 7.37–7.42 (m, 2H), 7.47 (d, 2H). MS (EI): *m*/*z* (%) 323 (97) [M^+], 267 (100).

5.1.3. Di-*tert*-butyl 5-benzyloxyindole-1,2-dicarboxylate (6). To a solution of **5** (1.07 g, 3.3 mmol) in dry THF (20 mL) was added di-*tert*-butyl dicarbonate (1.42 g, 6.6 mmol) and 4-dimethylaminopyridine (357 mg, 0.33 mmol). The mixture was stirred at room temperature for 2 h. Then the solvent was distilled off and the residue was purified by silica gel chromatography (hexane/ethyl acetate, 20:1) to give **6** as solid (1.31 g, 94%); mp 72 °C. ^{1}H NMR ($CDCl_3$): δ 1.59 (s, 9H), 1.63 (s, 9H), 5.10 (s, 2H), 6.97 (s, 1H), 7.07–7.11 (m, 2H), 7.33 (d, 1H), 7.37–7.41 (m, 2H), 7.45 (d, 2H), 7.93 (d, 1H). MS (EI): *m*/*z* (%) 423 (8) [M^+], 267 (100).

5.1.4. Di-*tert*-butyl 5-hydroxyindole-1,2-dicarboxylate (7). A solution of **6** (1.69 g, 4.0 mmol) in dry THF (60 mL) was treated with triethylamine (0.55 mL) and Pd/C (10% Pd, 550 mg). Then a balloon filled with hydrogen was attached. After stirring at room temperature for 1 h, the mixture was filtered and the filtrate evaporated to dryness. The residue was dissolved in ethyl acetate and filtered through a membrane filter (0.2 μm diameter). The filtrate was concentrated and the residue purified by chromatography on silica gel (hexane/ethyl acetate, 9:1) to afford **7** as solid (1.12 g, 84%); mp 165 °C. ^{1}H NMR ($CDCl_3$): δ 1.59 (s, 9H), 1.63 (s, 9H), 6.91–6.93 (m, 2H), 6.96 (d, 1H), 7.86 (d, 1H). MS (EI): *m*/*z* (%) 333 (8) [M^+], 77 (100).

5.1.5. Di-*tert*-butyl 5-(oxiran-2-ylmethoxy)indole-1,2-dicarboxylate (8). A solution of **7** (1.05 g, 3.2 mmol) in dry acetonitrile (55 mL) was treated under a nitrogen atmosphere with $KHCO_3$ (593 mg, 6.3 mmol) and tetrabutylammonium bromide (95 mg, 0.32 mmol). To the refluxing mixture epichlorohydrin (1.92 mL, 25 mmol) was added dropwise, and the resulting mixture was heated under reflux for additional 24 h. Then the solvent was distilled off and the residue was purified by silica gel chromatography (hexane/chloroform/ethyl acetate, 2:1:0.1) to give **8** as an oil (1.12 g, 92%). ^{1}H NMR ($CDCl_3$): δ 1.59 (s, 9H), 1.63 (s, 9H), 2.78 (dd, 1H), 2.91 (d, 1H), 3.39–3.41 (m, 1H), 3.98 (dd, 1H), 4.25 (dd, 1H), 6.97 (s, 1H), 7.03–7.05 (m, 2H), 7.93 (d, 1H). MS (EI): *m*/*z* (%) 289 (18) [M^+], 233 (100).

5.1.6. Di-*tert*-butyl 5-[2-hydroxy-3-(4-octylphenoxy)propoxy]indole-1,2-dicarboxylate (9). A mixture of **8** (450 mg, 1.2 mmol), 4-octylphenol (238 mg, 1.2 mmol) and 4-dimethylaminopyridine (14 mg, 0.12 mmol) was stirred at 40 °C for 48 h. The reaction mixture was subjected to silica gel chromatography (hexane/ethyl acetate, 9:1) to yield **9** as an oil (556 mg, 81%). ^{1}H NMR ($CDCl_3$): δ (ppm) 0.87 (t, 3H), 1.21–1.35 (m, 10H), 1.60 (s, 9H), 1.64 (s, 9H), 1.51–1.60 (m, 2H), 2.54 (t, 2H), 4.13–4.19 (m, 4H), 4.38–4.43 (m, 1H), 6.86 (d, 2H), 6.97 (s, 1H), 7.01–7.03 (m, 2H), 7.09 (d, 2H), 7.93 (d, 1H). MS (EI): *m*/*z* (%) 595 (5) [M^+], 233 (100).

5.1.7. Di-*tert*-butyl 5-[3-(4-octylphenoxy)-2-oxopropoxy]indole-1,2-dicarboxylate (10). Acetic anhydride (1.3 mL, 13 mmol) was added to dry DMSO (7 mL), and the mixture was stirred under a nitrogen atmosphere at room temperature for 10 min. Then this solution was added dropwise to a solution of **9** (200 mg, 0.34 mmol) in dry DMSO (7 mL). After being stirred at room temperature for 12 h, the reaction mixture was 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 with brine, dried (Na_2SO_4) and concentrated. The residue was purified by silica gel chromatography (hexane/ethyl acetate, 20:1) to give **10** as an oil (141 mg, 71%). ^{1}H NMR ($CDCl_3$): δ 0.88 (t, 3H), 1.22–1.35 (m, 10H), 1.59 (s, 9H), 1.64 (s, 9H), 1.52–1.62 (m, 2H), 2.55 (t, 2H), 4.86 (s, 2H), 4.92 (s, 2H), 6.84 (d, 2H), 6.97 (s, 1H), 6.99 (d, 1H), 7.07 (dd, 1H), 7.11 (d, 2H), 7.96 (d, 1H). MS (EI): *m*/*z* (%) 593 (1) [M^+], 437 (100).

5.1.8. 5-[3-(4-Octylphenoxy)-2-oxopropoxy]indole-2-carboxylic acid (11). To a solution of **10** (112 mg, 0.19 mmol) in dry CH_2Cl_2 (26 mL) was added trifluoroacetic acid (1.28 mL, 15 mmol), and the mixture was stirred at room temperature for 7 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 purified by silica gel chromatography (1. hexane/ethyl acetate, 1:3; 2. hexane/ethyl acetate/acetic acid, 1:3:0.01) to give **11** as solid (69 mg, 84%); mp 167 °C. ^{1}H NMR (DMSO-d_6): δ 0.82

(t, 3H), 1.15–1.31 (m, 10H), 1.43–1.52 (m, 2H), 2.54 (m, 2H), 4.96 (s, 2H), 4.97 (s, 2H), 6.82 (d, 2H), 6.93–6.96 (m, 2H), 7.05–7.07 (m, 3H), 7.33 (d, 1H), 11.63 (s, 1H). MS (EI): m/z (%) 437 (51) [M^+], 107 (100).

5.1.9. *tert*-Butyl 5-benzyloxy-1-methylindole-2-carboxylate (12). A mixture of **5** (1.5 g, 4.6 mmol), methyl-*p*-toluenesulfonate (947 mg, 5.1 mmol), tetrabutylammonium bromide (176 mg, 0.46 mmol), powdered NaOH (1.22 g, 30 mmol) and dry diethyl ether (100 mL) was stirred at room temperature for 18 h. After addition of water, the reaction mixture was extracted exhaustively with ethyl acetate. The combined organic phases were washed with 5% aqueous $NaHCO_3$-solution and brine, dried (Na_2SO_4) and concentrated. The residue was recrystallized from hexane/ethyl acetate to give **12** (1.3 g, 83%); mp 112 °C. ^{1}H NMR ($CDCl_3$): δ 1.61 (s, 9H), 4.03 (s, 3H), 5.10 (s, 2H), 7.10 (dd, 1H), 7.11–7.13 (m, 2H), 7.28 (d, 1H), 7.33 (d, 1H), 7.38–7.41 (m, 2H), 7.47 (d, 2H). MS (EI): *m*/*z* (%) 337 (79) [M^+], 190 (100).

5.1.10. *tert*-Butyl 5-hydroxy-1-methylindole-2-carboxylate (13). The benzyl ether group of **12** was cleaved applying the procedure described for the synthesis of **7**. Chromatography on silica gel (hexane/ethyl acetate, 4:1) gave **13** as solid (831 mg, 93%); mp 99 °C. ^{1}H NMR ($CDCl_3$): δ 1.60 (s, 9H), 4.01 (s, 3H), 6.94 (dd, 1H), 7.03 (d, 1H), 7.08 (s, 1H), 7.24 (d, 1H). MS (EI): *m*/*z* (%) 247 (38) [M^+], 191 (100).

5.1.11. *tert*-Butyl 1-methyl-5-(oxiran-2-ylmethoxy)indole-2-carboxylate (14). A solution of **13** (806 mg, 3.3 mmol) in dry acetonitrile (57 mL) was treated under a nitrogen atmosphere with KOH (183 mg, 3.3 mmol) and tetrabutylammonium bromide (98 mg, 0.33 mmol). To the refluxing mixture epichlorohydrin (1.98 mL, 26 mmol) was added dropwise, and the resulting mixture was heated under reflux for additional 2 h. Then the solvent was distilled off and the residue was purified by silica gel chromatography (hexane/ethyl acetate, 10:1) to give **14** as solid (695 mg, 70%); mp 120 °C. ^{1}H NMR ($CDCl_3$): δ 1.60 (s, 9H), 2.79 (dd, 1H), 2.92 (d, 1H), 3.37–3.42 (m, 1H), 4.01 (dd, 1H), 4.02 (s, 3H), 4.24 (dd, 1H), 7.03–7.07 (m, 2H), 7.12 (s, 1H), 7.27 (d, 1H). MS (EI): *m*/*z* (%) 303 (91) [M^+], 247 (100).

5.1.12. *tert*-Butyl 5-[2-hydroxy-3-(4-octylphenoxy)propoxy]-1-methylindole-2-carboxylate (15). A mixture of **14** (180 mg, 0.59 mmol), 4-octylphenol (124 mg, 0.59 mmol) and 4-dimethylaminopyridine (7 mg, 0.06 mmol) was stirred at 100 °C for 2.5 h. The reaction mixture was subjected to silica gel chromatography (hexane/ethyl acetate, 9:1) to yield **15** as solid (107 mg, 37%); mp 113 °C. ^{1}H NMR ($CDCl_3$): δ 0.88 (t, 3H), 1.22–1.36 (m, 10H), 1.53–1.62 (m, 2H), 1.61 (s, 9H), 2.54 (t, 2H), 2.62–2.67 (s, br), 4.03 (s, 3H), 4.12–4.22 (m, 4H), 4.38–4.45 (m, 1H), 6.86 (d, 2H), 7.03 (dd, 1H), 7.09–7.13 (m, 4H), 7.27 (d, 1H). MS (EI): *m*/*z* (%) 509 (100) [M^+].

5.1.13. *tert*-Butyl 1-methyl-5-[3-(4-octylphenoxy)-2-oxopropoxy]indole-2-carboxylate (16). Compound **15** was oxidized in a similar way as described above for the synthesis of **10**. The crude product was purified by silica gel chromatography (hexane/ethyl acetate, 20:1) to yield **16** as solid; mp 89 °C. ^{1}H NMR ($CDCl_3$): δ 0.88 (t, 3H), 1.22–1.35 (m, 10H), 1.51–1.61 (m, 2H), 1.61 (s, 9H), 2.55 (t, 2H), 4.03 (s, 3H), 4.88 (s, 2H), 4.91 (s, 2H), 6.84 (d, 2H), 7.02 (d, 1H), 7.08 (dd, 1H), 7.10 (d, 2H), 7.11 (s, 1H), 7.30 (d, 1H). MS (EI): *m*/*z* (%) 507 (50) [M^+], 451 (100).

5.1.14. 1-Methyl 5-[3-(4-octylphenoxy)-2-oxopropoxy]-indole-2-carboxylic acid (17). *tert*-Butyl ester **16** was hydrolyzed according to the procedure described above for the preparation of **11**. The crude product was purified by silica gel chromatography (hexane/ethyl acetate, 1:3) to yield **17** as solid; mp 148 °C. ^{1}H-NMR (DMSO-d_6): δ 0.81 (t, 3H), 1.17–1.28 (m, 10H), 1.44–1.56 (m, 2H), 2.48 (m, 2H), 3.98 (s, 3H), 4.97 (s, 2H), 4.98 (s, 2H), 6.83 (d, 2H), 6.98–7.02 (m, 2H), 7.04–7.08 (m, 3H), 7.45 (d, 1H). MS (EI): *m*/*z* (%) 451 (30) [M^+], 107 (100).

5.1.15. *tert*-Butyl 5-benzyloxy-1-propylindole-2-carboxylate (18). A mixture of **5** (675 mg, 2.09 mmol), *tert*-BuOK (264 mg, 2.3 mmol), and dry DMSO (7 mL) was heated at 110 °C for 15 min. After addition of a solution of 1-bromopropane (257 mg, 2.1 mmol) in dry DMSO (3 mL), heating was continued at the same temperature for an additional 30 min. The reaction mixture was allowed to cool, diluted with half-concentrated brine, and extracted exhaustively with diethyl ether. The combined organic layers were washed with water and brine, dried (Na_2SO_4), and concentrated. The residue was purified by silica gel chromatography (hexane/ethyl acetate, 20:1) to give **18** as solid (751 mg, 98%); mp 92 °C. ^{1}H NMR ($CDCl_3$): δ 0.92 (t, 3H), 1.60 (s, 9H), 1.75–1.86 (m, 2H), 4.47 (t, 2H), 5.09 (s, 2H), 7.08 (dd, 1H), 7.11–7.13 (m, 2H), 7.29 (d, 1H), 7.35–7.41 (m, 3H), 7.47 (d, 2H). MS (EI): *m*/*z* (%) = 365 (74) [M^+], 218 (100).

5.1.16. *tert*-Butyl 5-hydroxy-1-propylindole-2-carboxylate (19). The benzyl ether group of **18** (729 mg, 2.0 mmol) was cleaved applying the procedure described for the synthesis of **7**. Chromatography on silica gel (hexane/ethyl acetate, 9:1) gave **19** as solid (474 mg, 87%); mp 101 °C. ^{1}H NMR ($CDCl_3$): δ 0.91 (t, 3H), 1.60 (s, 9H), 1.77–1.84 (m, 2H), 4.46 (t, 2H), 6.92 (dd, 1H), 7.03 (d, 1H), 7.08 (s, 1H), 7.25 (d, 1H). MS (EI): *m*/*z* (%) 275 (42) [M^+], 190 (100).

5.1.17. *tert*-Butyl 5-[2-hydroxy-3-(4-octylphenoxy)propoxy]-1-propylindole-2-carboxylate (20). A mixture of **19** (424 mg, 1.5 mmol), 2-(4-octylphenoxymethyl)oxirane[7] (483 mg, 1.9 mmol) and 4-dimethylaminopyridine (15 mg, 0.12 mmol) was stirred at 100 °C for 12 h. The reaction mixture was purified by silica gel chromatography (hexane/ethyl acetate/methanol, 100:2:5) followed by reversed phase chromatography on RP18 phase eluting with acetonitrile to yield **20** as an oil (554 mg, 67%). ^{1}H NMR ($CDCl_3$): δ 0.86–0.93 (m, 6H), 1.23–1.33 (m, 10H), 1.53–1.62 (m, 2H), 1.61 (s, 9H), 1.78–1.85 (m, 2H), 2.54 (t, 2H), 4.10–4.22 (m, 4H), 4.39–4.43 (m, 1H), 4.47 (t, 2H), 6.86 (d, 2H), 7.01 (dd, 1H), 7.08–

7.14 (m, 4H), 7.28 (d, 1H). MS (EI): *m/z* (%) 537 (12) [M^+], 219 (100).

5.1.18. *tert*-Butyl 5-[3-(4-octylphenoxy)-2-oxopropoxy]-1-propylindole-2-carboxylate (21). Compound **20** (513 mg, 0.96 mmol) was oxidized in a similar way as described above for the synthesis of **10**. The crude product was purified by silica gel chromatography (hexane/ethyl acetate/methanol, 200:1:5) to yield **20** as an oil (221 mg, 43%). ^{1}H NMR ($CDCl_3$): δ 0.86–0.95 (m, 6H), 1.22–1.35 (m, 10H), 1.54–1.62 (m, 2H), 1.60 (s, 9H), 1.77–1.86 (m, 2H), 2.54 (t, 2H), 4.48 (t, 2H), 4.89 (s, 2H), 4.90 (s, 2H), 6.84 (d, 2H), 7.01 (d, 1H), 7.06 (dd, 1H), 7.08–7.13 (m, 3H), 7.31 (d, 1H). MS (EI): *m/z* (%) 535 (26) [M^+], 479 (100).

5.1.19. 5-[3-(4-Octylphenoxy)-2-oxopropoxy]-1-propylindole-2-carboxylic acid (22). *tert*-Butyl ester **21** was hydrolyzed for 3 h according to the procedure described above for the preparation of **11**. The crude product was purified by silica gel chromatography (chloroform/methanol, 99:1) to yield **22** as solid (84 mg, 53%); mp 136 °C. ^{1}H NMR ($CDCl_3$): δ (t, 3H), 0.94 (t, 3H), 1.21–1.36 (m, 10H), 1.53–1.62 (m, 2H), 1.79–1.90 (m, 2H), 2.55 (t, 2H), 4.53 (t, 2H), 4.88 (s, 2H), 4.94 (s, 2H), 6.85 (d, 2H), 7.04 (d, 1H), 7.09–7.15 (m, 3H), 7.35 (d, 1H), 7.36 (s, 1H). MS (EI): *m/z* (%) 479 (100) [M^+].

5.1.20. Synthesis of 23–28. Compounds **23**–**28** were prepared from **5** in a manner similar to that described for the synthesis of **18**, utilizing 1-bromohexane, benzyl bromide, the appropriate ω-bromoalkanoic acid *tert*-butylester and 4-bromomethylbenzoic acid *tert*-butylester, respectively. Purification was performed by the method indicated.

5.1.21. *tert*-Butyl 5-benzyloxy-1-hexylindole-2-carboxylate (23). Purification by silica gel chromatography (hexane/ethyl acetate, 20:1) gave **23** as an oil; ^{1}H NMR ($CDCl_3$): δ 0.87 (t, 3H), 1.25–1.40 (m, 6H), 1.60 (s, 9H), 1.70–1.80 (m, 2H), 4.50 (t, 2H), 5.09 (s, 2H), 7.10 (dd, 1H), 7.11–7.13 (m, 2H), 7.28 (d, 1H), 7.33–7.42 (m, 3H), 7.47 (d, 2H). MS (EI): *m/z* (%) 407 (26) [M^+], 260 (100).

5.1.22. *tert*-Butyl 1-benzyl-5-benzyloxyindole-2-carboxylate (24). Purification by silica gel chromatography (hexane/ethyl acetate, 50:1) gave **24** as solid; mp 141 °C. ^{1}H NMR ($CDCl_3$): δ 1.53 (s, 9H), 5.09 (s, 2H), 5.80 (s, 2H), 7.01–7.08 (m, 3H), 7.16–7.26 (m, 6H), 7.30–7.41 (m, 3H), 7.46 (d, 2H). MS (EI): *m/z* (%) 413 (69) [M^+], 357 (100).

5.1.23. *tert*-Butyl 5-benzyloxy-1-(*tert*-butoxycarbonylmethyl)indole-2-carboxylate (25). Purification by silica gel chromatography (hexane/ethyl acetate, 50:1) gave **25** as solid; mp 143 °C. ^{1}H NMR ($CDCl_3$): δ 1.43 (s, 9H), 1.58 (s, 9H), 5.09 (s, 2H), 5.15 (s, 2H), 7.09 (dd, 1H), 7.14 (d, 1H), 7.17 (s, 1H), 7.18 (d, 1H), 7.30–7.42 (m, 3H), 7.47 (d, 2H). MS (EI): *m/z* (%) 437 (100) [M^+].

5.1.24. *tert*-Butyl 5-benzyloxy-1-(3-*tert*-butoxycarbonylpropyl)indole-2-carboxylate (26). Purification by silica gel chromatography (hexane/ethyl acetate, 50:1) gave **26** as solid; mp 110 °C. ^{1}H NMR ($CDCl_3$): δ 1.45 (s, 9H), 1.60 (s, 9H), 2.03–2.12 (m, 2H), 2.23 (t, 2H), 4.56 (t, 2H), 5.09 (s, 2H), 7.08 (dd, 1H), 7.11 (d, 1H), 7.12 (s, 1H), 7.30–7.41 (m, 4H), 7.47 (d, 2H). MS (EI): *m/z* (%) 465 (71) [M^+], 176 (100).

5.1.25. *tert*-Butyl 5-benzyloxy-1-(5-*tert*-butoxycarbonylpentyl)indole-2-carboxylate (27). Purification by silica gel chromatography (hexane/ethyl acetate, 50:1) gave **27** as an oil; ^{1}H NMR ($CDCl_3$): δ 1.30–1.39 (m, 2H), 1.41 (s, 9H), 1.58–1.63 (m, 2H), 1.60 (s, 9H), 1.75–1.84 (m, 2H), 2.19 (t, 2H), 4.50 (t, 2H), 5.09 (s, 2H), 7.07 (dd, 1H), 7.11–7.13 (m, 2H), 7.27 (d, 1H), 7.31–7.42 (m, 3H), 7.47 (d, 2H). MS (EI): *m/z* (%) 493 (64) [M^+], 381 (100).

5.1.26. *tert*-Butyl 5-benzyloxy-1-(4-*tert*-butoxycarbonylbenzyl)indole-2-carboxylate (28). Purification by silica gel chromatography (hexane/ethyl acetate, 50:1) gave **28** as solid; mp 102 °C. ^{1}H NMR ($CDCl_3$): δ 1.53 (s, 9H), 1.55 (s, 9H), 5.08 (s, 2H), 5.83 (s, 2H), 7.00–7.05 (m, 3H), 7.11–7.17 (m, 2H), 7.21 (s, 1H), 7.29–7.41 (m, 3H), 7.45 (d, 2H), 7.86 (d, 2H). MS (EI): *m/z* (%) 513 (96) [M^+], 457 (100).

5.1.27. *tert*-Butyl 5-benzyloxy-1-(2-dimethylaminoethyl)indole-2-carboxylate (29). A mixture of **5** (880 mg, 2.72 mmol), *tert*-BuOK (343 mg, 3.0 mmol), and dry DMF (9 mL) was heated at 110 °C for 15 min. After addition of a solution of 2-chloro-*N*,*N*-dimethylethylamine HCl (431 mg, 3.0 mmol) and *tert*-BuOK (378 mg, 3.3 mmol) in dry DMF (5 mL), heating was continued at the same temperature for 3 h. The reaction mixture was allowed to cool, diluted with half-concentrated brine, and extracted exhaustively with diethyl ether. The combined organic layers were washed with water and brine, dried (Na_2SO_4), and concentrated. The residue was purified by silica gel chromatography (hexane/ethyl acetate/triethylamine, 40:2:1) to yield **29** as solid (724 mg, 68%); mp 96 °C. ^{1}H NMR ($CDCl_3$): δ 1.60 (s, 9H), 2.34 (s, 6H), 2.64 (t, 2H), 4.64 (t, 2H), 5.09 (s, 2H), 7.06–7.12 (m, 3H), 7.30–7.42 (m, 4H), 7.46 (d, 2H). MS (EI): *m/z* (%) 394 (26) [M^+], 58 (100).

5.1.28. Synthesis of compounds 30–36. Compounds **30** and **31** were prepared by the route used for the synthesis of **22**, starting from **23** and **24**, respectively. Compounds **32**–**36** were synthesized from **25** to **29** in the manner as described for the synthesis **17**. The target compounds were purified by the method indicated.

5.1.29. 1-Hexyl-5-[3-(4-octylphenoxy)-2-oxopropoxy]indole-2-carboxylic acid (30). Purification by silica gel chromatography (chloroform/methanol, 99:1) and recrystallization from ethyl acetate gave **30**; mp 127 °C. ^{1}H NMR ($CDCl_3$): δ 0.83–0.91(m, 6H), 1.20–1.40 (m, 16H), 1.53–1.62 (m, 2H), 1.75–1.83 (m, 2H), 2.55 (t, 2H), 4.55 (t, 2H), 4.89 (s, 2H), 4.94 (s, 2H), 6.84 (d, 2H), 7.02 (d, 1H), 7.08–7.16 (m, 3H), 7.35 (d, 1H), 7.37 (s,1H). MS (EI): *m/z* (%) 521 (100) [M^+].

5.1.30. 1-Benzyl-5-[3-(4-octylphenoxy)-2-oxopropoxy]-indole-2-carboxylic acid (31). Purification by silica gel chromatography (hexane/ethyl acetate, 4:1) yielded **31**; mp 137 °C. ^{1}H NMR ($CDCl_3$): δ 0.83–0.92 (m, 3H),

1.21–1.37 (m, 10H), 1.52–1.63 (m, 2H), 2.55 (t, 2H), 4.86 (s, 2H), 4.87 (s, 2H), 5.82 (s, 2H), 6.84 (d, 2H), 7.01–7.10 (m, 4H), 7.11 (d, 2H), 7.19–7.31 (m, 5H). MS (EI): *m/z* (%) 527 (100) [M^+].

5.1.31. 1-(Carboxymethyl)-5-[3-(4-octylphenoxy)-2-oxopropoxy]indole-2-carboxylic acid (32). Recrystallization from hexane/ethyl acetate gave **32**; mp 171 °C. ^{1}H NMR (DMSO-d_6): δ 0.83 (t, 3H), 1.18–1.31 (m, 10H), 1.45–1.55 (m, 2H), 2.47 (t, 2H), 4.98 (s, 2H), 4.99 (s, 2H), 5.25 (s, 2H), 6.84 (d, 2H), 7.02 (dd, 1H), 7.07 (d, 2H), 7.11 (d, 1H), 7.12 (s, 1H), 7.52 (d, 1H). MS (EI): *m/z* (%) 495 (7) [M^+], 107 (100).

5.1.32. 1-(Carboxypropyl)-5-[3-(4-octylphenoxy)-2-oxopropoxy]indole-2-carboxylic acid (33). Recrystallization from hexane/ethyl acetate gave **33**; mp 160 °C. ^{1}H NMR (DMSO-d_6): δ 0.83 (t, 3H), 1.16–1.25 (m, 10H), 1.45–1.56 (m, 2H), 1.84–1.96 (m, 2H), 2.16 (t, 2H), 2.49 (t, 2H), 4.53 (t, 2H), 4.98 (s, 4H), 6.83 (d, 2H), 7.01–7.11 (m, 5H), 7.52 (d, 1H). MS (EI): *m/z* (%) 523 (93) [M^+], 107 (100).

5.1.33. 1-(Carboxypentyl)-5-[3-(4-octylphenoxy)-2-oxopropoxy]indole-2-carboxylic acid (34). Recrystallization from hexane/ethyl acetate gave **34**; mp 149 °C. ^{1}H NMR (DMSO-d_6): δ 0.83 (t, 3H), 1.15–1.30 (m, 12H), 1.44–1.55 (m, 4H), 1.60–1.69 (m, 2H), 2.15 (t, 2H), 2.50 (t, 2H), 4.51 (t, 2H), 4.98 (s, 4H), 6.83 (d, 2H), 7.02 (dd, 1H), 7.04–7.09 (m, 4H), 7.49 (d, 1H). MS (EI): *m/z* (%) 551 (33) [M^+], 107 (100).

5.1.34. 1-(4-Carboxybenzyl)-5-[3-(4-octylphenoxy)-2-oxopropoxy]indole-2-carboxylic acid (35). Recrystallization from hexane/ethyl acetate gave **35**; mp 212 °C. ^{1}H NMR (DMSO-d_6): δ 0.78–0.81 (m, 3H), 1.12–1.25 (m, 10H), 1.40–1.51 (m, 2H), 2.45 (t, 2H), 4.95 (s, 2H), 4.97 (s, 2H), 5.86 (s, 2H), 6.80 (d, 2H), 6.97–7.06 (m, 5H), 7.11 (d, 1H), 7.20 (s, 1H), 7.39 (d, 1H), 7.79 (d, 2H). MS (EI): *m/z* (%) 571 (5) [M^+], 107 (100).

5.1.35. 1-(2-Dimethylaminoethyl)-5-[3-(4-octylphenoxy)-2-oxopropoxy]indole-2-carboxylic acid (36). Recrystallization from hexane/ethyl acetate gave **36**; mp 112 °C. ^{1}H NMR (DMSO-d_6): δ 0.80 (d, 3H), 1.17–1.23 (m, 10H), 1.41–1.51 (m, 2H), 2.46 (t, 2H) 2.85 (s, 6H), 3.37 (t, 2H), 4.81 (t, 2H), 4.96 (s, 2H), 4.99 (s, 2H), 6.81(d, 2H), 6.98–7.12 (m, 4H), 7.16 (s, 1H), 7.56 (d, 1H). MS (EI): *m/z* (%) 508 (1) [M^+], 107 (100).

5.1.36. 5-[2-Hydroxy-3-(4-octylphenoxy)propoxy]-1-methylindole-2-carboxylic acid (37). To a solution of **15** (30 mg, 0.06 mmol) in dry CH_2Cl_2 (3 mL) was added trifluoroacetic acid (0.4 mL, 4.7 mmol), and the mixture was stirred at room temperature for 6 h. Then the reaction mixture was concentrated to dryness. The residue was treated twice with hexane and the solvent was evaporated each time. The product was precipitated from hexane. ^{1}H NMR ($CDCl_3$): δ 0.88 (t, 3H), 1.20–1.35 (m, 10H), 1.57 (quint, 2H), 2.55 (t, 2H), 4.07 (s, 3H), 4.16–4.21 (m, 4H), 4.39–4.45 (m, 1H), 6.87 (d, 2H), 7.09–7.12 (m, 4H), 7.30–7.35 (m. 2H). MS (EI): *m/z* (%) 453 (50) [M^+], 191 (100).

5.1.37. 1-Carboxymethyl-5-[2-hydroxy-3-(4-octylphenoxy)propoxy]indole-2-carboxylic acid (38). Compound **38** was synthesized from the corresponding *tert*-butyl ester in the manner described for the synthesis of **37**. ^{1}H NMR ($CDCl_3$): δ 0.88 (t, 3H), 1.25–1.29 (m, 10H), 1.57 (quint, 2H), 2.54 (t, 2H), 4.13–4.19 (m, 4H), 4.37–4.43 (m, 1H), 5.24 (s, 2H), 6.86 (d, 2H), 7.08–7.12 (m, 4H), 7.22 (d, 1H), 7.43 (s, 1H). MS (EI): *m/z* (%) 497 (75) [M^+], 191 (100).

5.2. Evaluation of the target compounds

5.2.1. General. The HPLC system applied for measuring enzyme inhibition, metabolic stability and solubility 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.

Acetonitrile (HPLC grade) and phosphoric acid (85%) were obtained from Baker (Deventer, Netherlands). Dimethylsulfoxide (DMSO) (p.a.) and magnesium chloride hexahydrate (ultra) were purchased from Fluka (Buchs, Switzerland). Dihydronicotinamide adenine dinucleotide phosphate tetrasodium salt (NADPH) was acquired from Roth (Karlsruhe, Germany). 5,8,11,14-Eicosatetraynoic acid (ETYA) and arachidonyl trifluoromethyl ketone ($AACOCF_3$) were got from Biomol (Hamburg, Germany). Phosphate-buffered saline tablets for the preparation of phosphate-buffered saline (0.1 M, pH 7.4), 12-*O*-tetradecanoylphorbol-13-acetate (TPA) and nordihydroguaiaretic acid (NDGA) were obtained from Sigma (Deisenhofen, Germany). Methanol (HPLC grade) was purchased from Acros Organics (Geel, Belgium). Calcium chloride, EDTA-Na_2 and $(NH_4)_2HPO_4$ were acquired from Merck (Darmstadt, Germany). Human buffy coat was a gift from the German Red Cross (Münster, Germany). The internal standard 3-(4-decyloxyphenyl)propanoic acid was synthesized according to a published procedure.[23]

5.2.2. Inhibition of cPLA$_2\alpha$

5.2.2.1. Assay with the isolated enzyme. The ability of test compounds to inhibit cPLA$_2\alpha$ isolated from human platelets was performed as previously described.[12] Briefly, sonicated covesicles consisting of 1-stearoyl-2-arachidonoyl-*sn*-glycero-3-phosphocholine (0.2 mM) and 1,2-dioleoyl-*sn*-glycerol (0.1 mM) were used as substrate. Enzyme reaction was terminated 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 NDGA as oxygen scavenger. cPLA$_2\alpha$ activity was determined by measuring 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 cleaning up the samples by solid-phase extraction.

5.2.2.2. Cellular assay. Preparation of human blood platelets: Buffy coat (about 50 mL) was centrifuged in three 50 ml polypropylene tubes at 2000*g* for 2 min and the platelet-rich supernatants were carefully separated by aspiration. The obtained platelet-rich fraction

was then centrifuged at 1000*g* for 15 min. The pellet was resuspended in a mixture of phosphate-buffered saline (0.1 M, pH 7.4) and 3.7% aqueous EDTA–Na_2 (97:3, v/v). The volume of this mixture was twice the volume of the platelet-rich supernatant obtained before. The suspension was centrifuged at 1000*g* for 15 min and the platelets were resuspended in phosphate-buffered saline (0.1 M, pH 7.4). The final cell concentration was adjusted to about 10^8 cells/mL (0.1 mL of this cell suspension diluted with 0.9 mL of phosphate buffered saline (0.1 M, pH 7.4) gave an absorbance of 0.12 at 800 nm). The platelets were stored at +4 °C until used.

Incubation procedure: A DMSO solution of ETYA (1.19 mg/mL) (5 μL; final concentration 10 μM), a DMSO solution of the test compound (5 μL)-in the case of the controls DMSO (5 μL) was taken instead-and platelet suspension (1.59 μL) were preincubated in a shaking water bath at 37 °C. After 5 min, a $CaCl_2$ solution (10 mM in 0.8% w/v saline) (0.4 μL) was added and preincubation was carried on for further 5 min. Then the cells were stimulated by adding a DMSO solution of 12-*O*-tetradecanoylphorbol-13-acetate (TPA) (0.49 mg/mL) (5 μL; final concentration 2 μM) and the incubation was continued for 60 min at 37 °C. The enzyme reaction was terminated by the addition of a mixture of acetonitrile, methanol and 0.1 M aqueous EDTA–Na_2 solution (16:15:1, v/v/v) (2 mL) containing NDGA as oxygen scavenger (6 μg/2 mL) and 3-(4-decyloxyphenyl)propanoic acid as internal standard (0.8 μg/2 mL). After cooling for 10 min in an ice bath, the samples were centrifuged at 1000*g* for 10 min at +4 °C. The supernatants were stored at −20 °C.

Sample preparation and HPLC-analysis: The supernatants were diluted with water (15 mL) and then applied to a Bakerbond spe™ octadecyl reversed phase solid phase extraction column (500 mg, 19 mL wide mouth, JT Baker, Deventer, Netherlands), which had successively been washed before with methanol (10 mL), water (5 mL) and 0.1% aqueous EDTA-Na_2 (5 mL) solution. After washing the column with water (5 mL), the adsorbed arachidonic acid was eluted with methanol (3 × 1 mL). The eluate was diluted with water (5 mL) and then subjected to HPLC. As stationary phase a RP18 multospher 100 column (3 μm, 3.0 mm (ID) × 125 mm) with a RP18 multospher 100 guard column (5 μm, 3.0 mm (ID) × 20 mm) (CS-chromatographie service, Langerwehe, Germany) was applied. The mobile phase consisted of acetonitrile/aqueous $(NH_4)_2HPO_4$ (10 mM) adjusted to pH 7.4 with phosphoric acid (85%) (50:50, v/v). Oven temperature was set to 30 °C. The flow rate was 0.3 mL/min and the injected sample volume was 0.6 mL. The detection wavelength was 200 nm. After each run the column was washed with 1.0 mL methanol. Control incubations in the absence of the stimulant TPA were carried out in parallel and used to calculate the specific hydrolysis.

5.2.3. Metabolic stability. Rat-liver microsomes were prepared by differential centrifugation as described previously[11] and adjusted with phosphate-buffered saline (0.1 M, pH 7.4) to a protein content of 5.7 mg/mL. An aliquot of the microsome suspension (198 μL) was added to a solution of test compound in DMSO (2 μL, 5 mM). The final concentration of the test compound was 20 μM. After a 5 min preincubation at 37 °C, the metabolic reactions were started by addition of a solution of NADPH (12 mM) and $MgCl_2$ (5 mM) in phosphate buffered saline (0.1 M, pH 7.4) (300 μL). The incubation was terminated after 30 min by addition of acetonitrile (500 μL) and cooling at 0 °C. After further 15 min, the samples were centrifuged at 2000*g* for 5 min. The supernatants were stored in closed vials at −20 °C until subjection to HPLC. Reference incubations without NADPH were performed and analyzed in the same way. Separation was achieved on a Waters Symmetry C_{18} analytical column (3.5 μm, 4.6 mm (ID) × 75 mm) or a Phenomenex Aqua C_{18} column (3 μm, 4.6 mm (ID) × 75 mm). A 30 μL volume of each sample was injected onto the HPLC system. The temperature of the autosampler and the column oven were maintained at 20 °C. The mobile phase consisted of mixture of solvent A (acetonitrile/water/phosphoric acid (85%), 80:20:0.1, v/v/v) and solvent B (acetonitrile/water/phosphoric acid (85%), 50:50:0.1, v/v/v). The ratio of the two solvents applied depended on the lipophilicity of the test compounds. The flow rate was 0.7 mL/min. The effluents were monitored at 240 nm. The degree of metabolism of the parent compounds was calculated from the ratio of the peak areas obtained in the presence and absence of NADPH.

LC–MS experiments were performed to elucidate the structure of the respective main metabolites. A 75 μL (100 μL at MS^n investigations) volume of the assay solution was injected onto a Kromasil 100-5C_{18} column (5 μm, 2 mm (ID) × 60 mm, inlet filter 2 μm) at a flow rate of 0.4 mL/min. The mobile phase consisted of aqueous ammonium formiate solution (20 mM)/acetonitrile 80:20 (v/v) (A) and aqueous ammonium formiate solution (20 mM)/acetonitrile 15:85 (v/v) (B). The initial composition was 65 % A. The gradient was programmed linearly to 0% A over 12 min and held for 3 min. Finally, the gradient was linearly increased to 65% A over 1 min and held for 4 min. The HPLC system consisting of a Waters 2690 separation module was coupled on-line to the LCQ® ion trap mass spectrometer from Thermo-Finnigan via an ESI-interface. The ESI-source voltage was set to 3 kV, the sheath gas flow was adjusted to 80 arbitrary units and the auxiliary gas flow-rate was set to 0 arbitrary units. The temperature of the heated capillary was 200 °C, the tube lens offset was adjusted to 55 V and the capillary voltage set to −6 V. The mass spectra (*m*/*z* 250–1200) were recorded in negative mode. MS^n-mode experiments were accomplished with an isolation width of *m*/*z* 3 and normalized collision energy of 35–40%. For the simultaneous UV-detection a Waters UV/Vis-detector model 2487 was used. The detection wavelength was set to 254 nm.

In every mass chromatogram besides the expected $[M-H]^-$ ions for the ketones, hydrate ions of these substances with $[M+18-H]^-$ were observed (Fig. 2). For the main metabolites, which eluted without exception between hydrate and the ketone form of the test compound (=parent peak), $[M+2-H]^-$ ions were detected.

Therefore, it can be assumed that the main metabolites of the activated ketones are their corresponding alcohols. Additionally, in case of **17** and **32** the reference alcohols **37** and **38** were synthesized and spiked to the incubation probes leading to an increase of peak intensity of the main metabolites. Furthermore, LC/MS^3 investigations were performed for these two alcohols by fragmenting the particular $[M+2-H]^-$ ions. After isolation and collision of these ions, stable daughter ions appeared at $[M+2-44-H]^-$, which could be further fragmented to identical product ions in incubation and spiked reference probes, respectively.

5.2.4. Solubility. Thermodynamic solubility was determined in a similar way as previously described.[16] To 1 mg of the test compound was added phosphate-buffered saline (0.1 M, pH 7.4) (2 mL). The mixture was sonicated for 10 min (Bandelin Sonorex TK52 sonication bath) and then shaken for 20 h at room temperature (20–23 °C). After centrifugation (4000*g*, 10 min) at room temperature, about 1 mL of the supernatant was separated. To 200 μL of this solution were added acetonitrile (250 μL) and 0.1 M aqueous phosphoric acid (50 μL). An aliquot of this solution (5–100 μL) was subjected to HPLC analysis. Separation was achieved on a Waters Symmetry C_{18} analytical column (3.5 μm, 4.6 mm (ID) × 75 mm) or a Phenomenex Aqua C_{18} column (3 μm, 4.6 mm (ID) × 75 mm). The mobile phase consisted of mixture of solvent A (acetonitrile/water/phosphoric acid (85%), 80:20:0.1, v/v/v) and solvent B (acetonitrile/water/phosphoric acid (85%), 50:50:0.1, v/v/v). The ratio of the two solvents applied depended on the lipophilicity of the test compounds. The flow rate was 0.7 mL/min. The effluents were monitored at 240 nm. The amount of test compound dissolved was calculated using a calibration curve. The calibration solutions were prepared by mixing obtained standard stock solutions of the test compounds in DMSO (2 μL) with phosphate buffered saline (0.1 M, pH 7.4) (198 μL), acetonitrile (250 μL) and 0.1 M aqueous phosphoric acid (50 μL). Before HPLC analysis, the solutions were equilibrated for at least 30 min at 20 °C.

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