Bioorganic & Medicinal Chemistry Letters 19 (2009) 2107–2111

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

1-(5-Carboxyindol-1-yl)propan-2-ones as inhibitors of human cytosolic phospholipase $A_2\alpha$: Synthesis and properties of bioisosteric benzimidazole, benzotriazole and indazole analogues

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ARTICLE INFO

Article history:
Received 12 February 2009
Revised 4 March 2009
Accepted 5 March 2009
Available online 10 March 2009

Keywords:
Indole
Bioisosters
Cytosolic phospholipase $A_2\alpha$ inhibitors
Metabolic stability
Solubility

ABSTRACT

The indole ring systems of the cytosolic phospholipase $A_2\alpha$ ($cPLA_2\alpha$) inhibitor 1-[3-(4-octylphenoxy)-2-oxopropyl]indole-5-carboxylic acid (**2**) and the isomeric 6-carboxylic acid (**3**) were replaced by benzimidazole, benzotriazole and indazole scaffolds, respectively. The effect of the structural variations on $cPLA_2\alpha$ inhibitory potency, metabolic stability and solubility was studied. The lead **2** and the indazole-5-carboxylic acid **28** were the metabolically most stable compounds in an assay with rat liver microsomes, while the benzimidazole-5-carboxylic acid derivative **13** possessed the best water solubility (22 μg/mL at pH 7.4). The indazole-5-carboxylic acid **28** revealed the highest $cPLA_2\alpha$ inhibitory potency of the compounds in this series. With an IC_{50}-value of 0.005 μM it was about sevenfold more active than the lead **2**.

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

Despite several potent inhibitors of $cPLA_2\alpha$ having been developed, such as thiazolidinediones of Shionogi,[6,7] benzhydrylindoles of Wyeth[8,9] and 1,3-diaryloxypropan-2-ones of AstraZeneca (**1**, AR-C70484XX),[10] only the compounds of Wyeth have undergone clinical development.[11] Recently, we have published a series of $cPLA_2\alpha$ inhibitors with indole scaffold like **2** and **3**, structurally related to **1** (Fig. 1).[12–14]

Structure–activity relationship studies on indole-5-carboxylic acid **2** have revealed that substitution of the indole 3-position strongly influences the activity of the compound.[12] We wanted to investigate the effect of a replacement of the carbon at position 3, and also at position 2 of the indole scaffold by nitrogen. Therefore, the heteroanalogous benzimidazole, benzotriazole and indazole derivatives of **2** were synthesized and evaluated for $cPLA_2\alpha$ inhibitory potency. Furthermore, the stability of the electrophilic ketone groups of these compounds against metabolic reduction was investigated in a test system applying rat liver microsomes, since conversion of the ketone moiety of **2** to an alcohol functionality causes loss of $cPLA_2\alpha$ inhibition.[15,16] Finally, also the aqueous solubility of the target compounds was measured, since a sufficient dissolution is one important constraint for oral bioavailability.

The synthesis of the benzimidazole analogue of **2** started from methyl 1*H*-benzimidazole-5-carboxylate (**4**) (Scheme 1). N-Alkyl-

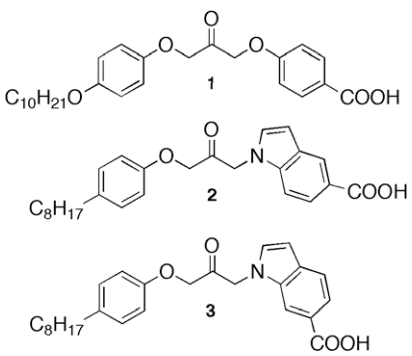

Figure 1.

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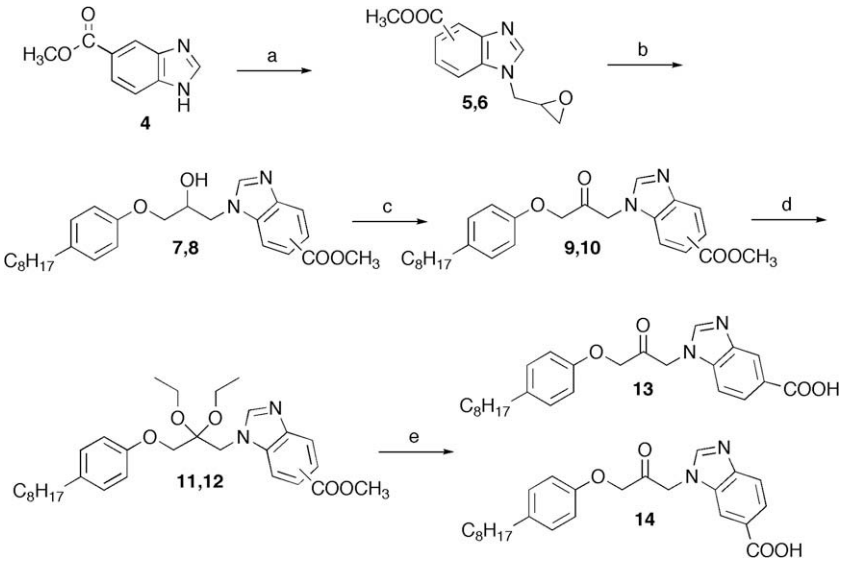
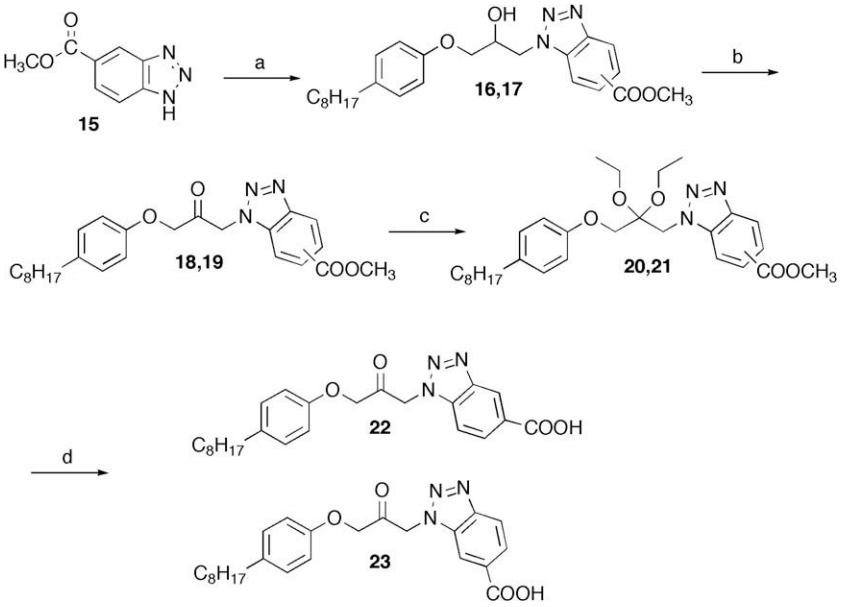
doi:10.1016/j.bmcl.2009.03.019



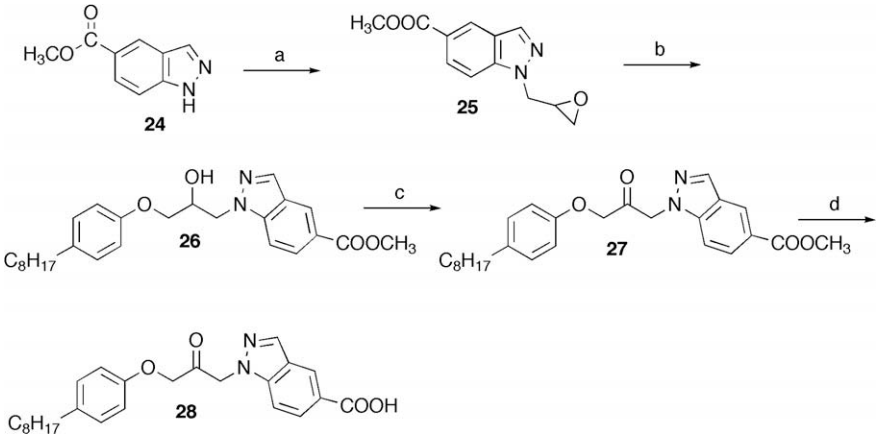
Scheme 1. Reagents and conditions: (a) Epichlorohydrin, KOH, $Bu_4N^+Br^-$, rt, 2 h; (b) 4-octylphenol, 4-dimethylaminopyridine, 120 °C, 30 min; (c) Dess–Martin periodinane reagent, CH_2Cl_2, rt, 3 h; (d) triethyl orthoformate, ethanol, H_2SO_4, reflux, 12 h; (e) (1) aq NaOH, methanol, reflux, 1,5 h, (2) aq HCl, THF, reflux, 3 h.

ation with epichlorohydrin yielded two isomeric products, which were shown to be the benzimidazole-5-carboxylic and -6-carboxylic ester derivatives **5** and **6** (ratio about 1:1 evaluated by ^1H NMR spectroscopy). Since **5** and **6** as well as the following methyl ester derivatives **7**, **8**–**11**, **12** could not be separated successfully by chromatography on silica gel, mixtures of the isomers were applied as reactants in the subsequent reaction steps. Regioselective opening of the oxirane ring of **5**, **6** was achieved without solvent in the presence of catalytic amounts of 4-dimethylaminopyridine. Oxidation of the resulting alcohol intermediates **7**, **8** to the desired ketones **9**, **10** was carried out with Dess–Martin periodinane reagent. Next the ketone groups of **9**, **10** were acetalized with triethyl orthoformate in the presence of catalytic amounts of H_2SO_4. The methyl ester groups of obtained compounds **11**, **12** were saponified with aqueous NaOH. Finally, the acetal protecting group was removed by aqueous HCl in THF to yield a mixture of benzimidazole-5- and -6-carboxylic acid derivatives **13** and **14**. These two acids could be separated by reversed phase HPLC. The structures of **13** and **14** were assigned by NOE experiments. Irradiation of the N–CH_2–CO protons of **13** gave NOE-enhancements of the doublet signal of the proton at C-7 of the benzimidazole at 7.17–7.19 ppm, confirming that **13** is the benzimidazole-5-carboxylic acid derivative. The structure of the other isomer conclusively was established as being benzimidazole-6-carboxylic acid **14**.

Reaction of methyl 1*H*-benzotriazole-5-carboxylate (**15**) with 2-(4-octylphenoxymethyl)oxirane[12] also led to a mixture of two isomers (**16**, **17**) (Scheme 2). From these, the target benzotriazole-5- and -6-carboxylic acids **22** and **23**[17] were afforded, using the reaction sequence described above for the synthesis of **13** and **14** from **7** and **8**. Separation of the target compounds **22** and **23** was performed by normal phase chromatography on silica gel.



Scheme 2. Reagents and conditions: (a) 2-(4-Octylphenoxymethyl)oxirane, NaH, DMF, 90 °C, 2 h; (b) Dess–Martin periodinane reagent, CH_2Cl_2, rt, 3 h; (c) triethyl orthoformate, ethanol, H_2SO_4, reflux, 6.5 h; (d) (1) aq NaOH, methanol, reflux, 1.5 h, (2) aq HCl, THF, reflux, 8 h.



Scheme 3. Reagents and conditions: (a) Epichlorohydrin, KOH, $Bu_4N^+Br^-$, rt, 2 h; (b) 4-octylphenol, 4-dimethylaminopyridine, 120 °C, 45 min; (c) Dess–Martin periodinane reagent, CH_2Cl_2, rt, 3 h; (d) aq KOH, ethanol, rt, 18 h.

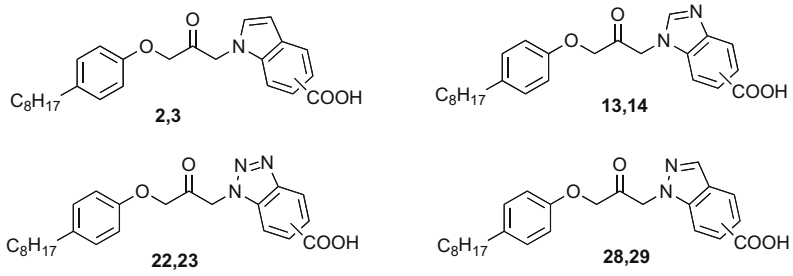
Scheme 3 outlines the synthesis of indazole-5-carboxylic acid **28**. The 2-oxopropyl substituent was introduced in position 1 of methyl indazole-5-carboxylate (**24**) applying the reaction sequence described for the synthesis of **9** and **10** (Scheme 1). The methyl ester group of obtained compound **27** was hydrolyzed with aqueous KOH in ethanol to give the target compound **28**.[18] Indazole-6-carboxylic acid derivative **29** was synthesized in the same way starting from methyl indazole-6-carboxylate.

Indole-5-carboxylic acid **2** has been found to be a potent inhibitor of cPLA$_2\alpha$ with an IC_{50}-value of 0.035 μM against the enzyme isolated from human platelets.[12] In the course of further structure–activity relationship studies, one aim was to replace the indole scaffold of **2** by benzimidazole, benzotriazole, and indazole, respectively. During the synthesis of the benzimidazole and the benzotriazole bioisosters of **2**, not only the desired 5-carboxylic acids **13** and **22**, but also the isomeric 6-carboxylic acids **14** and **23** were obtained. Therefore, we included carboxylic acid derivatives with carboxylic acid group in position 6 of the heterocycles in our investigations too.

Evaluation of the inhibitory activity against cPLA$_2\alpha$[19] showed that introduction of a nitrogen atom at position 3 of indole-5-carboxylic acid **2** led to decrease of activity. With an IC_{50} of 0.15 μM the benzimidazole-5-carboxylic acid **13** is about fourfold less potent than indole-5-carboxylic acid **2** (Table 1). In contrast, shifting the nitrogen from position 3 to position 2 significantly increased enzyme inhibitory potency, as seen by the inhibition data of the indazole-5-carboxylic acid **28** (IC_{50}: 0.005 μM). The benzotriazole-5-carboxylic acid **22** (IC_{50}: 0.026 μM) again has about the same activity as the indole lead **2**. Here the negative effect of nitrogen in position 3 seems to be compensated by the positive effect of nitrogen in position 2 of the heterocycle.

The structure–activity relationships of the 6-carboxylic acid series synthesized differ remarkably from that of the corresponding 5-carboxlic acids. Here, as well nitrogen in position 3 as nitrogen in position 2 led to a significant increase of enzyme inhibition. While the indole-6-carboxylic acid **3** possesses an IC_{50} of 1.1 μM, the IC_{50}-values of the benzimidazole- and indazole-6-carboxylic acids **14** and **29** are 0.085 μM and 0.026 μM, respectively. Concomitant

Table 1
cPLA2α-inhibitory potency, metabolic stability and thermodynamic solubility



Compd	Position of the COOH-group	Inhibition of cPLA$_2\alpha$ IC_{50}[a] (μM)	Metabolic stability[b] (%)	Thermodynamic solubility[c] (μg/mL)
2	5	0.035	65 ± 5	<1
3	6	1.1	39	<1
13	5	0.15	49	22 ± 8
14	6	0.085	40	7 ± 0.8
22	5	0.026	43	<1
23	6	0.016	44	2 ± 0.2
28	5	0.005	64	<1
29	6	0.026	36	5 ± 0.8

[a] Values are the means of at least two independent determinations, errors are within ±20%; IC_{50}-value of reference inhibitor **1** (AR-C70484XX): 0.011 μM.[10,12]
[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 **2**: mean ± standard deviation, $n = 5$.
[c] Mean ± standard deviation, $n = 3$, in case of values <1 μg/mL: $n = 2$.

introduction of two nitrogen atoms in position 3 and 2 had an additive effect. Thus, the activity of the benzotriazole-6-carboxylic acid **23** (IC_{50}: 0.016 μM) is higher than that of **14** and **29**, respectively.

Structure–activity relationship studies have revealed that an important pharmacophoric element of **2** is the activated ketone moiety.[12,15] Its reduction to an alcohol results in a loss of cPLA$_2$α inhibitory potency. Like other compounds with electrophilic ketone groups,[20,21] **2** is metabolically susceptible to keto reduction.[14,16] To investigate metabolic stability of the ketone group of the new compounds, their metabolisation by rat liver microsomes was also studied.[19] After incubation of the microsomes in presence of NADPH still about 65% of the indole-5-carboxylic acid **2** could be measured by HPLC and UV-detection in comparison with reference incubations in absence of NADPH. The main metabolite formed was the corresponding inactive alcohol as shown by LC–MS experiments performed as described recently.[14,16] Indazole-5-carboxylic acid **28** showed about the same metabolic stability (64%) as the corresponding indole derivative **2**, while all other compounds evaluated were less stable (Table 1). At the end of the metabolic reactions only 30–50% of these substances were detectable yet. Comparing the metabolism of the 5-carboxylic acids with that of the 6-carboxylic acids, one striking difference could be observed. In case of the 5-carboxylic acid derivatives **2**, **13**, **22** and **28**, LC–MS experiments showed that the corresponding alcohols of the compounds were the main metabolites. Contrary, in case of the 6-carboxylic acid derivatives **3**, **14**, **23** and **29** only small amounts of their alcohol forms could be detected. Instead several more polar metabolites occurred.

Due to the two long acyl chains, the phospholipid substrates of cPLA$_2$α possess a substantial lipophilicity. Therefore it can be expected that inhibitors, which shall bind competitively to the active site of the enzyme, must possess similar properties. This assumption is confirmed by the fact that all known cPLA$_2$α inhibitors with pronounced potency bear larger lipophilic residues,[6–10,12] 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 aqueous solubility of all new compounds under thermodynamic conditions[19,22] by equilibrating the solids in phosphate buffer (pH 7.4) for 20 h at room temperature, separating non-soluble material by centrifugation and measuring the soluble aqueous concentration by HPLC. Both indolecarboxylic acid derivatives (**2**,**3**) possess a water solubility of less than 1 μg/mL (Table 1). Replacement of the indole scaffold by a benzimidazole resulted in more soluble compounds (**13**: 22 μg/mL; **14**: 7 μg/mL). With solubility values of less than 1 μg/mL, benzotriazole- and indazole-5-carboxylic acids **22** and **28** again have only poor water solubility. The dissolution values of the benzotriazole- and indazole-6-carboxylic **23** (2 μg/mL) and **29** (5 μg/mL) slightly exceed the amount of 1 μg/mL. Solubility guidelines for drugs under development are given by Lipinski and co-workers.[22] According to those, compounds with mid-range permeability and average potency should possess a minimum thermodynamic solubility of 50 μg/mL. With a solubility of 22 μg/mL, **13** comes closest to this limit.

In conclusion, we have studied the effect of the replacement of the indole scaffold of **2** and **3** by benzimidazole, benzotriazole and indazole on cPLA$_2$α inhibitory potency, metabolic stability and solubility. The compounds with the highest metabolic stability were indole-5- and indazole-5-carboxylic acids **2** and **28**. Substitution of indole by benzimidazole increased water solubility significantly. The indazole-5-carboxylic acid **28** showed the highest activity against cPLA$_2$α. With an IC_{50}-value of 0.005 μM, **28** is about sevenfold more active than the lead **2** and represents one of the most potent inhibitors of cPLA$_2$α known today.[3–5] The aim of further studies will be to develop derivatives of these compounds, concomitantly possessing high cPLA$_2$α-inhibitory potency, adequate water solubility and good stability against metabolic reduction of the pharmacophoric ketone group.

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17. *Final step of the synthesis of 1-[3-(4-octylphenoxy)-2-oxopropyl]-1H-benzotriazole-6-carboxylic acid* **23:** To solution of a mixture of **20** and **21** (170 mg, 0.33 mmol) in methanol (10 mL) was added a solution of NaOH (1.0 g) in water (120 mL). The mixture was heated under reflux for 1.5 h. After acidifying with 6 M HCl, the mixture was concentrated to about 20 mL, treated with THF (30 mL) and heated under reflux for additional 8 h. After concentrating until some precipitates appeared, the reaction mixture was extracted exhaustively with ethyl acetate. The organic layers were dried (Na_2SO_4) and the solvent was evaporated. The two isomers **22** and **23** were separated and purified by chromatography on silica gel (0.015–0.040 mm) eluting with hexane/ethyl acetate/formic acid (1. 8:2:0.5; 2. 7:3:0.5) to yield **22** (67 mg) and **23** (12 mg) as solids; mp 211–212 °C and 174–176 °C, respectively. Compound **23**: ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 0.82 (t, J = 6.8 Hz, 3H), 1.21–1.24 (m, 10H), 1.50–1.52 (m, 2H), 2.48–2.49 (m, 2H), 5.12 (s, 2H), 6.11 (s, 2H), 6.90 (d, J = 8.6 Hz, 2H), 7.10 (d, J = 8.6 Hz, 2H), 7.94 (d, J = 8.6 Hz, 1H), 8.13 (d, J = 8.6 Hz, 1H), 8.52 (s, 1H), 13.26 (s, broad, 1H). 1D NOE (600 MHz, DMSO-d_6): δ (ppm) $^1H_{(irr)}$/$^1H_{(res)}$ 6.11 ($COCH_2$ N)/8.52 (benzotriazole H-7) and 5.12 (OCH_2CO); 8.52 (benzotriazole H-7)/6.11 ($COCH_2$ N). MS (ESI+): m/z 424 $[M+H]^+$.
18. *Final step of the synthesis of 1-[3-(4-octylphenoxy)-2-oxopropyl]indazole-5-carboxylic acid* **28**: A solution of **27** (68 mg, 0.16 mmol) in ethanol (6 mL) was treated with a solution of 10% aqueous KOH (2 mL). The mixture was stirred at room temperature for 18 h. After acidification with 1 M HCl, the reaction mixture was 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, 8:2:0.1) yielded **28** (28 mg, 43%). For further purification, an aliquot of the product (11 mg) was subjected to semi-preparative RP-HPLC applying acetonitrile/H_2O/formic acid (800:200:0.4) as mobile phase. The eluates were concentrated under reduced pressure until most of the acetonitrile was removed. Freeze drying of the remaining solution gave **28** (8 mg) as a solid; mp 160–162 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.83 (t, J = 6.8 Hz, 3H), 1.16–1.28 (m, 10H), 1.45–1.55 (m, 2H), 2.45–2.50 (m, 2H), 5.01 (s, 2H), 5.68 (s, 2H), 6.85 (d, J = 8.6 Hz, 2H), 7.09 (d, J = 8.6 Hz, 2H), 7.63 (d, J = 8.9 Hz, 1H), 7.93 (dd, J = 8.8 Hz and J = 1.5 Hz, 1H), 8.27–8.28 (m, 1H), 8.44–8.45 (m, 1H). MS (ESI+): m/z 423 $[M+H]^+$.
19. *Inhibition of cPLA$_2$α:* The target compounds were evaluated in an assay applying cPLA$_2$α isolated from human platelets.[23] 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. *Metabolic stability:* Test compounds were incubated with rat liver microsomes under aerobic conditions in absence and presence of the cofactor NADPH as described previously.[14] 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 LC/MS analysis.[14,16] *Water Solubility:* Water solubility was determined experimentally according to published procedures.[14,24] Briefly, sodium phosphate buffer (pH 7.4) was added to a test compound, and the suspension obtained was equilibrated by sonication (10 min) 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 regression curve.

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