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1-Indol-1-yl-propan-2-ones and related heterocyclic compounds as dual inhibitors of cytosolic phospholipase $A_2\alpha$ and fatty acid amide hydrolase

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ABSTRACT

Cytosolic phospholipase $A_2\alpha$ (cPLA$_2\alpha$) and fatty acid amide hydrolase (FAAH) are enzymes, which have emerged as attractive targets for the development of analgetic and anti-inflammatory drugs. We recently reported that 1-[3-(4-octylphenoxy)-2-oxopropyl]indole-5-carboxylic acid (**10**) and related compounds are inhibitors of cPLA$_2\alpha$. Since cPLA$_2\alpha$ and FAAH possess several common structural features, we now screened this substance series together with some new derivatives for FAAH inhibition. Some of the assayed compounds proved to be selective cPLA$_2\alpha$ inhibitors, while others showed high FAAH and moderate cPLA$_2\alpha$ inhibitory potency. Furthermore, several derivatives were favorably active against both enzymes and, therefore, could represent agents, which have improved analgetic and anti-inflammatory qualities in comparison with selective cPLA$_2\alpha$ and FAAH inhibitors.

1. Introduction

In mammalian organism, derivatives of arachidonic acid play important roles as algesic and pro-inflammatory as well as analgesic and anti-inflammatory mediators. On the one hand, oxidation products of arachidonic acid such as prostaglandin E_2 and leukotriene B_4 formed via the arachidonic acid cascade are involved in the pathophysiology of pain and inflammation.[1] On the other hand, the arachidonic acid amide anandamide generated via the endocannabinoid pathway has analgetic and anti-inflammatory properties.[2]

The key enzyme in the formation of oxidized derivatives of arachidonic acid is cytosolic phospholipase $A_2\alpha$ (cPLA$_2\alpha$).[3,4] This enzyme provides arachidonic acid for prostaglandin and leukotriene synthesis by cleaving membrane phospholipids at the *sn*-2 position. Therefore, cPLA$_2\alpha$ is considered as a target for treatment of pain and inflammatory diseases.[5,6] First-generation cPLA$_2\alpha$ inhibitors were analogues of arachidonic acid with the COOH group replaced by $COCF_3$ (AACOCF$_3$, **1**)[7,8] (Fig. 1) or $CH_2PO(OCH_3)F$ (MAFP, **2**).[9] Compounds with high in vitro cPLA$_2\alpha$ inhibitory potency reported later are benzhydrylindoles from Wyeth,[10] thiazolidinedione compounds from Shionogi (**3**),[11] and propan-2-ones, such as **4** (AR-C70484XX), from AstraZeneca (Fig. 1).[12]

An important enzyme in the endocannabinoid metabolism is fatty acid amide hydrolase (FAAH), which rapidly cleaves the analgetic and anti-inflammatory lipid mediator anandamide into arachidonic acid and ethanolamine.[13–15] Inhibition of FAAH is supposed to enhance the action of anandamide. Therefore, like cPLA$_2\alpha$ inhibitors, inhibitors of FAAH may represent new agents against pain and inflammation.[16,17] In the past years many potent inhibitors of FAAH have been found such as the carbamate **5** (URB 597) and the α-ketoheterocycle **6** (PHOP) (Fig. 1).[18–29]

FAAH and cPLA$_2\alpha$ have several common features.[30–32] Both enzymes are serine hydrolases cleaving arachidonoyl residues from their appropriate substrates. Following formation of the enzyme–substrate complexes, the active site serines of the enzymes attack the arachidonoyl-ester and -amide, respectively, resulting in the formation of oxyanions. These tetrahedral transition states are stabilized by 'oxyanion holes' built by the backbone amide groups of two glycine residues of each enzyme. Upon collapse of the oxyanions, serine-arachidonoyl intermediates are generated, which finally are hydrolyzed by water. Many inhibitors of FAAH and cPLA$_2\alpha$, respectively, bind covalently to the active site serines of the enzymes. So-called serine-trap inhibitors of FAAH are the carbamate **5** and the α-ketoheterocycle **6**.[18–22] Known cPLA$_2\alpha$ inhibitors acting in this way are the trifluoromethylketone **1**, the fluorophosphonate **2** and the activated acetone derivative **4**.[7–9,12] Interestingly, **1** and **2**, initially developed as cPLA$_2\alpha$ inhibitors, also inhibit FAAH activity.[33–36]

Recently, we have described a series of cPLA$_2\alpha$ inhibitors such as 5-carboxyindol-1-ylpropan-2-one **10** (Fig. 1) structurally related to **4**.[37–39] On account of the described similarities between cPLA$_2\alpha$ and FAAH, we have now tested our cPLA$_2\alpha$ inhibitors for FAAH inhibition too. This was of special interest since dual inhibitors of

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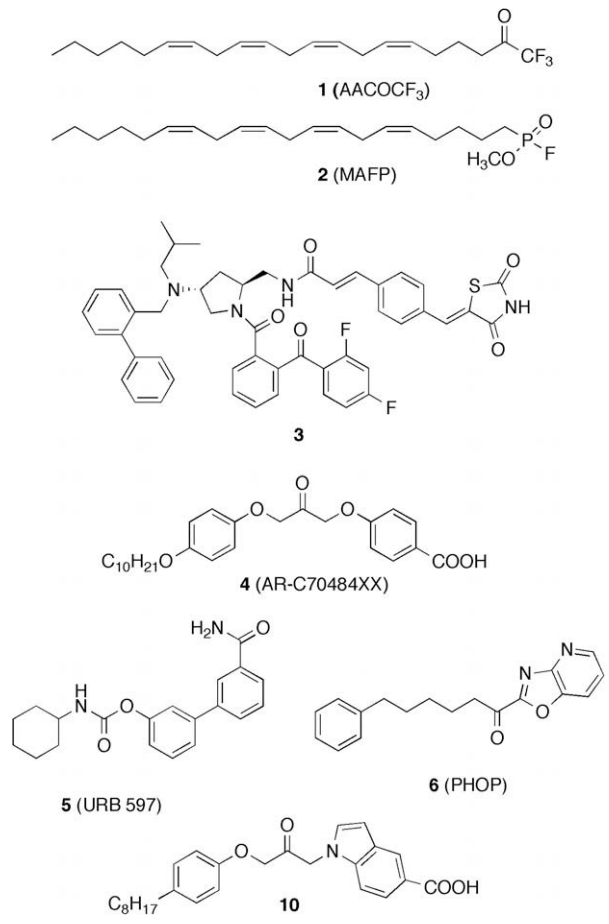
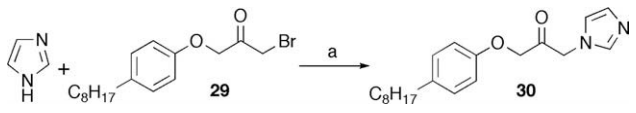


Figure 1.

cPLA$_2\alpha$ and FAAH could have additional benefits in comparison with selective ones, because they would inhibit the formation of pro-inflammatory and the degradation of anti-inflammatory lipid mediators simultaneously.

2. Chemistry

The syntheses of most of the compounds investigated have already been described.[37–39] The imidazole derivative **30** (Scheme 1) and the benzimidazole derivative **44** were prepared by reaction of imidazole and benzimidazole, respectively, with 1-bromo-3-(4-octylphenoxy)propan-2-one (**29**), obtained by Dess–Martin oxidation of 1-bromo-3-(4-octylphenoxy)propan-2-ol.[37]



Scheme 1.

Pyrazole-4-carboxylic acid **38** was synthesized by the route outlined in Scheme 2. Ethyl pyrazole-4-carboxylate was coupled with 2-(4-octylphenoxy)methyloxirane[37] by heating without solvent. Oxidation of the resulting alcohol intermediate **36** to the ketone **37** was carried out with Dess–Martin periodinane reagent. Finally, the ethyl ester group of **37** was hydrolyzed with aqueous KOH in ethanol to give the target compound **38**.

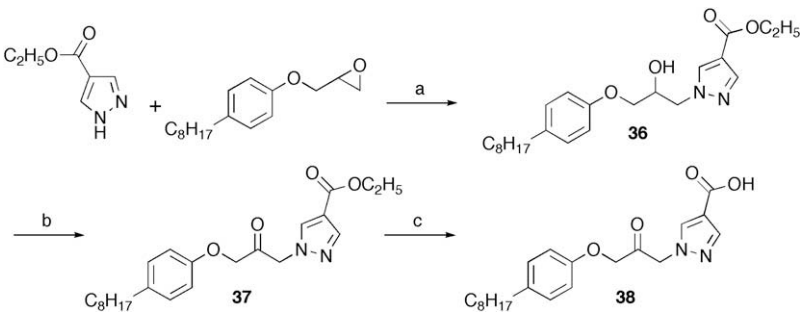
Reaction of methyl pyrazole-3-carboxylate and methyl imidazole-4-carboxylate with 2-(4-octylphenoxy)methyloxirane led to the formation of two regioisomers in each case, namely methyl pyrazole-3- and -5-carboxylate derivatives **33a** and **33b** and methyl imidazole-4- and -5-carboxylate derivatives **40a** and **40b**. The corresponding isomers were separated by silica gel chromatography and further reacted as described for the synthesis of **38** (Scheme 2) to afford the test compounds **35**, **39**, **41** and **42**.

The preparation of the benzotriazole derivative **47** is shown in Scheme 3. Reaction of benzotriazole with epichlorohydrin led to the oxiranylmethyl-substituted compound **45**. Opening of the epoxy ring of **45** with 4-octylphenol was achieved without solvent in the presence of catalytic amounts of 4-dimethylaminopyridine. Oxidation of the resulting alcohol intermediate **46** to the target ketone **47** was carried out with Dess–Martin reagent.

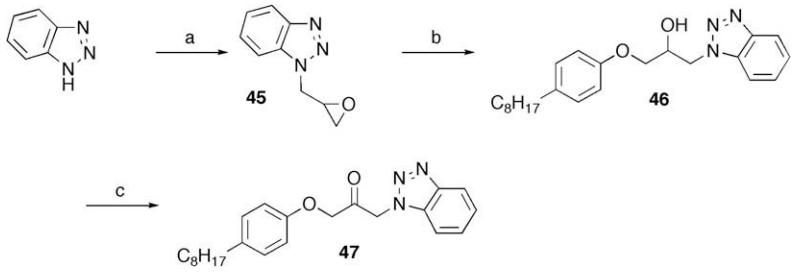
3. Biological evaluation

All target compounds were tested for cPLA$_2\alpha$ inhibition applying cPLA$_2\alpha$ isolated from human platelets.[40] 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.

For evaluation of FAAH inhibition, microsomes from rat brain served as enzyme source. *N*-(2-Hydroxyethyl)-4-pyren-1-ylbutanamide was used as substrate in combination with the micelle forming agent Triton X-100.[41] The FAAH activity was determined by measuring the amount of 4-pyren-1-ylbutanoic acid released by the enzyme during 45 min with reversed-phase HPLC and fluorescence-detection.cPLA$_2\alpha$ and FAAH are enzymes that work at the surface of the lipophilic cell membranes. A possible problem of assays with such enzymes is that a test compound could inhibit the enzyme not by binding to its active site but merely by altering the lipophilic 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 should be kept low.[42] Thus, in our enzyme assays the concentration of the substances forming the lipophilic substrate phase was chosen



Scheme 2.



Scheme 3.

significantly higher (300 μM in case of cPLA$_2$α and about 3000 μM in case of FAAH) than the maximal concentration of inhibitor (10 μM).

4. Results and discussion

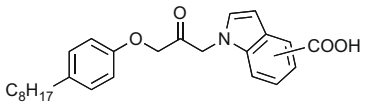
The known dual cPLA$_2$α/FAAH inhibitor **1** exhibited IC$_{50}$ values of about 3 μM against both enzymes in our assays. The fluorophosphonate **2** blocked cPLA$_2$α (IC$_{50}$ = 0.64 μM) and especially FAAH (IC$_{50}$ = 0.023 μM) with considerable higher potency (Table 1). The investigation of the regioisomeric 1-(2-oxopropyl)indolecarboxylic acids **7**–**11** has revealed, that the derivative with the carboxylic acid group at position 5 (**10**) is a potent cPLA$_2$α inhibitor (IC$_{50}$ = 0.035 μM).[37] Screening **10** for FAAH inhibition now showed that it also possesses a substantial activity against this enzyme. With an IC$_{50}$ of 2.8 μM it is about as active as the reference **1**. In contrast, the other isomers are significantly less active or even inactive.

The carboxylic acid group of **10** is important for both cPLA$_2$α and FAAH inhibition. This can be seen by the fact that the unsubstituted indole derivative **12** and the compounds with a methyl, chloro, methoxy, cyano, methoxycarbonyl and formyl group in position 5 (**13–18**) are no or only poor inhibitors of the enzymes (Table 2). Best tolerated is the replacement of the 5-carboxylic acid by a carboxamide-moiety. In this case inhibitory potency against cPLA$_2$α and FAAH is reduced only two to threefold.

Introduction of substituents at position 3 of the indole-5-carboxylic acid **10** created striking different effects on cPLA$_2$α and FAAH inhibitory potency. While this structural variation led to several compounds with significant higher cPLA$_2$α inhibitory potency such as the cyano, formyl, acetyl and methoxycarbonyl derivatives **22–25**, it concurrently resulted in a loss of FAAH inhibition in all cases (Table 3).

Like the indole **12**, the analogous pyrrole **27** did not inhibit either of the enzymes. However, introduction of a second nitrogen in the pyrrole heterocycles led to compounds, which affected cPLA$_2$α as well as FAAH activity. While the IC$_{50}$ values of the pyrazole **28** lay in the micromolar range, the imidazole derivative **30**

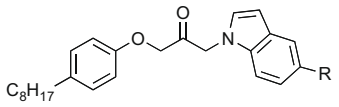
Table 1
Inhibition of FAAH and cPLA$_2$α



Compd	Position of the carboxylic acid moiety at the indole ring	Inhibition of FAAH IC$_{50}$[a] (μM)	Inhibition of cPLA$_2$α IC$_{50}$[a] (μM)
7	2	n.a.[b]	n.a.[b]
8	3	n.a.[b]	>10[c]
9	4	>10[c]	>10[c]
10	5	2.8	0.035
11	6	n.a.[b]	1.1
1 (AACOCF$_3$)		3.7	2.3
2 (MAFP)		0.023	0.64

[a] Values are the means of at least two independent determinations; errors are within ±20%.
[b] Not active at 10 μM.
[c] Indicates about 40% inhibition of enzyme activity at 10 μM.

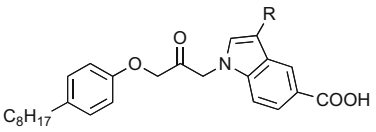
Table 2
Inhibition of FAAH and cPLA$_2$α



Compd	R	Inhibition of FAAH IC$_{50}$[a] (μM)	Inhibition of cPLA$_2$αIC$_{50}$[a] (μM)
10	COOH	2.8	0.035
12	H	n.a.[b]	n.a.[b]
13	CH_3	n.a.[b]	n.a.[b]
14	Cl	n.a.[b]	n.a.[b]
15	OCH_3	>10[c]	n.a.[b]
16	CN	>10[d]	>10[e]
17	$COOCH_3$	n.a.[b]	>10[f]
18	CHO	>10[g]	4.3
19	$CONH_2$	6.4	0.12

[a] Values are the means of at least two independent determinations; errors are within ±20%.
[b] Not active at 10 μM.
[c] Indicates 22% of enzyme activity at 10 μM.
[d] Indicates 42% of enzyme activity at 10 μM.
[e] Indicates 25% of enzyme activity at 10 μM.
[f] Indicates 36% of enzyme activity at 10 μM.
[g] Indicates 45% of enzyme activity at 10 μM.

Table 3
Inhibition of FAAH and cPLA$_2$α



Compd	R	Inhibition of FAAH IC$_{50}$[a] (μM)	Inhibition of cPLA$_2$α IC$_{50}$[a] (μM)
10	H	2.8	0.035
20	$C(CH_3)_3$	n.a.[b]	0.34
21	Cl	n.a.[b]	0.11
22	CN	n.a.[b]	0.015
23	CHO	n.a.[b]	0.016
24	$COCH_3$	n.a.[b]	0.012
25	$COOCH_3$	n.a.[b]	0.005
26	COOH	n.a.[b]	0.89

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

exhibited even submicromolar inhibition values (IC_{50} = 0.76 μM and 0.50 μM, respectively) (Table 4).

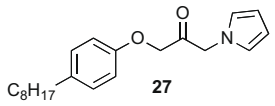
Introduction of carboxylic acid groups in the pyrrole, pyrazole and imidazole ring, respectively, of **27**, **28** and **30** produced similar effects on cPLA$_2$α and FAAH inhibitory potency (Table 5). A carboxy group in α-position to the N1-nitrogen led to compounds, which were inactive or only weakly active (IC_{50} >10 μM) (**31**, **39**, **42**). Shifting the acid group into α-position, however, increased inhibitory activity in all cases. The pyrazole-4-carboxylic acid **38** was the most active cPLA$_2$α inhibitor (IC_{50} = 1.8 μM) and FAAH inhibitor (IC_{50} = 0.11 μM) of this series (Table 5).

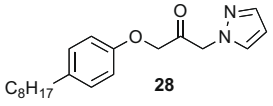
Replacement of the indole heterocycle of **12** by indazole (**43**), benzimidazole (**44**) and benzotriazole (**47**) successively increased inhibitory potency against FAAH (Table 6). While indole **12** was even inactive at 10 μM, the benzotriazole derivative **47** was highly potent showing an IC_{50} of 0.047 μM. Thus, as in case of the pyrrole **27**, introduction of nitrogens into the five-membered ring resulted in more active FAAH inhibitors. Inhibition of cPLA$_2$α was not effected as dramatically as FAAH inhibition by this structural modification. Only the benzotriazole **47** possessed a moderate activity against cPLA$_2$α (IC_{50} = 2.2 μM).

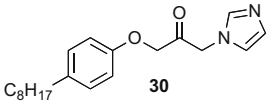
Substitution of indole **12**, indazole **43**, benzimidazole **44** and benzotriazole **47** with a carboxy group in position 5 or 6 of the heterocyclic ring system elevated cPLA$_2$α inhibitory potency in each case significantly (Table 7). Contrary, the effect of this structural variation on FAAH inhibition was inconsistent. A carboxy group in position 6 of the indole, indazole and benzimidazole ring, respectively, reduced activity of the compounds (**11**, **49**, **51**), while such a substitution in 5-position increased it (**10**, **48**, **50**). In case of benzotriazole **47**, however, a three to fourfold reduction of potency against FAAH occurs with the introduction of a carboxy group in position 5 or 6 (**52**,**53**).

Finally, we also tested some other inhibitors of cPLA$_2$α and FAAH, respectively, for inhibition of these enzymes (Table 8). The known potent FAAH inhibitors **5** and **6** did not inhibit cPLA$_2$α at the highest concentration tested (10 μM). Vice versa, the thiazolidinedione cPLA$_2$α inhibitor **3** was inactive against FAAH. However,

Table 4
Inhibition of FAAH and cPLA$_2$α



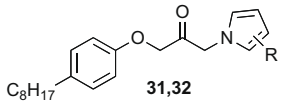


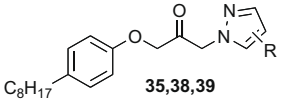


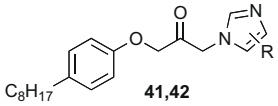
Compd	Inhibition of FAAH IC_{50}[a] (μM)	Inhibition of cPLA$_2$α IC_{50}[a] (μM)
27	n.a.	n.a.
28	2.0	>10[b]
30	0.76	0.50

[a] Values are the means of at least two independent determinations, errors are within ±20%.
[b] Indicates about 43% inhibition of enzyme activity at 10 μM.

Table 5
Inhibition of FAAH and cPLA$_2$α



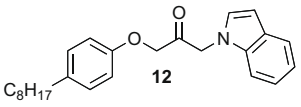


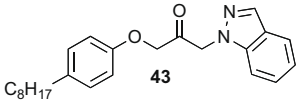


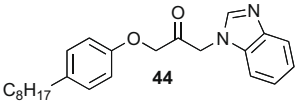
Compd	R	Inhibition of FAAH IC_{50}[a] (μM)	Inhibition of cPLA$_2$α IC_{50}[a] (μM)
31	2-COOH	n.a.[b]	n.a.[b]
32	3-COOH	3.6	5.0
35	3-COOH	>10[c]	8.2
38	4-COOH	0.11	1.8
39	5-COOH	n.a.[b]	>10[d]
41	4-COOH	1.9	5.3
42	5-COOH	n.a.[b]	>10[e]

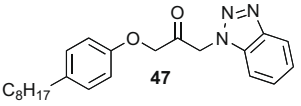
[a] Values are the means of at least two independent determinations; errors are within ±20%.
[b] Not active at 10 μM.
[c] Indicates 39% inhibition of enzyme activity at 10 μM.
[d] Indicates 21% inhibition of enzyme activity at 10 μM.
[e] Indicates 18% inhibition of enzyme activity at 10 μM.

Table 6
Inhibition of FAAH and cPLA$_2$α





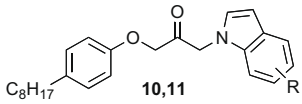


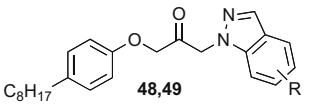


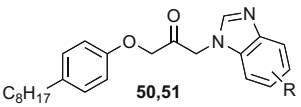
Compd	Inhibition of FAAH IC_{50}[a] (μM)	Inhibition of cPLA$_2$α IC_{50}[a] (μM)
12	n.a.[b]	n.a.[b]
43	5.0	n.a.[b]
44	1.0	>10[c]
47	0.047	2.2

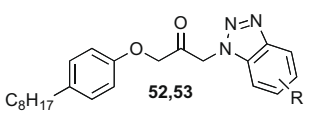
[a] Values are the means of at least two independent determinations; errors are within ±20%.
[b] Not active at 10 μM.
[c] Indicates 36% inhibition of enzyme activity at 10 μM.

Table 7
Inhibition of FAAH and cPLA$_2$α









Compd	R	Inhibition of FAAH IC_{50}[a] (μM)	Inhibition of cPLA$_2$α IC_{50}[a] (μM)
10	5-COOH	2.6	0.035
11	6-COOH	n.a.[b]	1.1
48	5-COOH	0.16	0.005
49	6-COOH	>10[c]	0.026
50	5-COOH	0.61	0.15
51	6-COOH	3.1	0.085
52	5-COOH	0.14	0.026
53	6-COOH	0.20	0.016

[a] Values are the means of at least two independent determinations; errors are within ±20%.
[b] Not active at 10 μM.
[c] Indicates 23% inhibition of enzyme activity at 10 μM.

Table 8
Inhibition of FAAH and cPLA$_2\alpha$ by reference inhibitors

Compd	Inhibition of FAAH IC_{50}[a] (μM)	Inhibition of cPLA$_2\alpha$ IC_{50}[a] (μM)
1 ($AACOCF_3$)	3.7	2.3
2 (MAFP)	0.023	0.64
3 (Thiazolidinedione derivative)	n.a.[b]	0.031
4 (AR-C70484XX)	0.059	0.011
5 (URB597)	0.24	n.a.[b]
6 (PHOP)	0.009	n.a.[b]

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

the potent cPLA$_2\alpha$ inhibitor **4** of AstraZeneca (IC_{50} = 0.011 μM) also blocked FAAH activity with high potency (IC_{50} = 0.059 μM).

Besides anandamide, 2-arachidonoyl glycerol (2-AG) is the second important endocannabinoid. FAAH is also able to hydrolyze this compound.[43] However, the main enzyme for inactivation of 2-AG has been suggested to be monoacylglycerol lipase (MAGL). Like cPLA$_2\alpha$ and FAAH, MAGL is a serine hydrolase, which is strongly inhibited by methylarachidonoyl fluorophosphonate (**2**).[43] The propane-2-ones presented in Tables 1–7, therefore, were evaluated for inhibition of MAGL additionally. A test system analogous to that for measuring FAAH inhibition was applied, utilizing a synthetic pyrene labelled fluorescence substrate (here: 1,3-dihydroxypropan-2-yl-4-pyren-1-ylbutanoate) as well.[44] We found that none of the compounds was active at the highest test concentration of 10 μM. In the same way, propane-2-one **4** did not show MAGL inhibitory potency. In contrast, the dual cPLA$_2\alpha$ and FAAH **2** exhibited an IC_{50} of 0.062 μM in our assay.

In conclusion, we have found that several of the indol-1-ylpropan-2-ones and structurally related heterocyclic compounds recently published as cPLA$_2\alpha$ inhibitors[37–39] are also inhibitors of FAAH. Inhibitory potency against the two enzymes can be controlled by the substitution pattern of the heterocycles. For high inhibition of cPLA$_2\alpha$, a carboxylic acid group at the heterocyclic part of the molecules with a certain distance to the activated ketone is necessary. On the contrary, such a carboxylate moiety is not required unconditionally for a strong interaction with FAAH. Introduction of substituents like chloro, acetyl, cyano and methoxycarbonyl in position 3 of the indole-5-carboxylic acid **10** leads to a selective cPLA$_2\alpha$ inhibition. The pyrazole-4-carboxylic acid **38** and the benzotriazole derivative **47** are potent FAAH inhibitors exhibiting only moderate activity against cPLA$_2\alpha$. The indazole-5-carboxylic acid **48** and the benzotriazole-5- and 6-carboxylic acids **52** and **53** can be considered as favorably active dual cPLA$_2\alpha$/FAAH inhibitors. The IC_{50}s of these compounds for cPLA$_2\alpha$ inhibition lie in the low nanomolar range and their IC_{50}s against FAAH is lower or equal than 0.20 μM. The best dual cPLA$_2\alpha$/FAAH inhibitor tested so far, however, was reference compound **4**, published by Connolly and co-workers from AstraZeneca (IC_{50} = 0.011 μM and 0.059 μM, respectively (Table 8).

5. Experimental

5.1. Chemistry

5.1.1. General

Column chromatography was performed on Merck Silica Gel 60, 0.040–0.063 mm (230–400 mesh). Melting points were determined on a Büchi B-540 apparatus 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) and electrospray ionization (ESI), respectively. Purity of the new target compounds was determined by HPLC with UV-detection at 254 nm. A cyano phase (LiChrospher 100 CN, 5 μm, 3.0 mm (ID) × 250 mm, Merck, Darmstadt, Germany) was applied eluting with an isohexane/THF gradient containing 0.1% trifluoroacetic acid (**35**, **38**, **39**, **47**), an isohexane/THF gradient containing 0.1% ethyldiisopropylamine (**30**, **44**) or acetonitrile/water/H_3PO_4 (85%) (70:30:0.1, v/v/v) (**41**, **42**). The flow rate was 0.5 mL/min. All target compounds showed purities ⩾97%.

5.1.2. 1-Bromo-3-(4-octylphenoxy)propan-2-one (29)

A solution of 1-bromo-3-(4-octylphenoxy)propan-2-ol[37] (110 mg, 0.32 mmol) in dry CH_2Cl_2 (3 mL) was treated with Dess–Martin periodinane reagent (204 mg, 0.48 mmol) and stirred under a nitrogen atmosphere at room temperature for 4 h. Then a solution of sodium thiosulfate (0.4 g) in saturated sodium bicarbonate solution (15 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 (hexane/ethyl acetate, 8:2) to yield **29** as a solid (90 mg, 82%); mp 50 °C. ^{1}H NMR ($CDCl_3$) δ 0.88 (t, J = 7.1 Hz, 3H), 1.26–1.30 (m, 10H), 1.53–1.67 (m, 2H), 2.55 (t, J = 7.8 Hz, 2H), 4.17 (s, 2H), 4.76 (s, 2H), 6.62 (d, J = 8.6 Hz, 2H), 7.11 (d, J = 8.6 Hz, 2H). MS (EI): m/z (%) 342 (23), 340 (24) [M^+], 241 (100).

5.1.3. 1-Imidazol-1-yl-3-(4-octylphenoxy)propan-2-one (30)

Under a nitrogen atmosphere a solution of imidazole (17 mg, 0.25 mmol) in dry DMF (3 mL) was treated with K_2CO_3 (35 mg, 0.25 mmol). At 50 °C a solution of **29** (77 mg, 0.23 mmol) in dry DMF (2 mL) was added dropwise and heating was continued at this temperature for 1 h. After addition of half-saturated brine (50 mL), the reaction mixture was extracted exhaustively with ethyl acetate. The combined organic layers were washed with half-saturated brine, dried (Na_2SO_4) and the solvent was evaporated. After chromatography on silica gel (CH_2Cl_2/methanol, 95:5), the obtained product was dissolved in a small amount of acetonitrile and precipitated by addition of water. The solvents were removed by freeze drying to afford **30** as a solid (29 mg, 35 %); mp 92 °C. ^{1}H NMR ($CDCl_3$) δ 0.88 (t, J = 7.0 Hz, 3H), 1.26–1.27 (m, 10H), 1.51–1.61 (m, 2H), 2.57 (t, J = 7.4 Hz, 2H), 4.66 (s, 2H), 5.11 (s, 2H), 6.84 (d, J = 8.6 Hz, 2H), 6.87–6.89 (m, 1H), 7.12–7.13 (m, 1H), 7.15 (d, J = 8.7 Hz, 2H), 7.45 (s, 1H). MS (EI): m/z (%) 329 (1) [M^+], 82 (100).

5.1.4. Methyl 1-[2-hydroxy-3-(4-octylphenoxy)propyl]pyrazole-3-carboxylate (33a) and -5-carboxylate (33b)

A mixture of methyl pyrazole-3-carboxylate (283 mg, 2.2 mmol) and 2-(4-octylphenoxymethyl)oxirane (577 mg, 2.2 mmol) was heated at 100 °C for 6 h. The obtained melt was dissolved in toluene and chromatographed on silica gel (CH_2Cl_2/methanol, 98:2) to yield **33a** (188 mg, 22%) and **33b** (29 mg, 3 %) as solids.

Compound **33a**: Mp 92 °C. ^{1}H NMR ($CDCl_3$): δ 0.87 (t, J = 7.0 Hz, 3H), 1.26–1.29 (m, 10H), 1.55–1.59 (m, 2H), 2.53 (t, J = 7.4 Hz, 2H), 3.03 (d, J = 5.1 Hz, 1H), 3.92 (s, 3H), 3.89–3.92 (m, 2H), 4.36–4.46 (m, 2H), 4.50 (dd, J = 12.7 Hz and 2.5 Hz, 1H), 6.80 (dd, J = 8.6 Hz, J = 2.0 Hz, 2H), 6.82 (d, J = 2.4 Hz, 1H), 7.08 (d, J = 8.6 Hz, 2H), 7.52 (d, J = 2.4 Hz, 1H). MS (EI): m/z (%) 388 (1) [M^+], 183 (100).

Compound **33b**: Mp 42 °C. ^{1}H NMR ($CDCl_3$): δ 0.88 (t, J = 7.0 Hz, 3H), 1.26–1.30 (m, 10H), 1.54–1.58 (m, 2H), 2.53 (t, J = 7.4 Hz, 2H), 3.58 (d, J = 5.5 Hz, 1H), 3.86 (s, 3H), 3.91 (dd, J = 9.6 Hz and 5.4 Hz, 1H), 4.01 (dd, J = 9.6 Hz and 5.0 Hz, 1H), 4.36–4.43 (m, 1H), 4.86–4.89 (m, 2H), 6.82 (dd, J = 9.0 and 2.4 Hz, 2H), 6.87 (d, J = 2.0 Hz, 1H), 7.08 (dd, J = 9.0 Hz and 2.4 Hz, 2H), 7.55 (d, J = 2.0 Hz, 1H). MS (EI): m/z (%) 388 (2) [M^+], 183 (100).

5.1.5. Methyl 1-[3-(4-octylphenoxy)-2-oxopropyl]pyrazole-3-carboxylate (34)

Compound **33a** (95 mg, 0.24 mmol) was oxidized with Dess–Martin periodinane (156 mg, 0.37 mmol) as described above for the synthesis of **29**. The crude product was purified by silica gel chromatography (CH_2Cl_2/methanol, 98:2) to yield **34** as a solid (91 mg, 96%); mp 105 °C. ^{1}H NMR ($CDCl_3$): δ 0.87 (t, J = 7.0 Hz, 3H), 1.26–1.29 (m, 10H), 1.55–1.59 (m, 2H), 2.55 (t, J = 7.4 Hz, 2H), 3.92 (s, 3H), 4.65 (s, 2H), 5.39 (s, 2H), 6.82 (dd, J = 8.6 Hz and 2.3 Hz, 2H), 6.90 (d, J = 2.4 Hz, 1H), 7.13 (dd, J = 9.0 Hz and 2.4 Hz, 2H), 7.46 (d, J = 2.3 Hz, 1H). NOE ($CDCl_3$): δ $^1H_{irr}$/δ $^1H_{res}$ 7.46 (pyrazol-5-C*H*)/6.90 (pyrazol-4-C*H*), 5.39 (propyl-1-CH_2); 6.90 (pyrazol-4-C*H*)/7.46 (pyrazol-5-C*H*); 5.39 (propyl-1-CH_2)/7.46 (pyrazol-5-C*H*), 6.82 (phenyl-2-C*H*, phenyl-6-C*H*), 4.65 (propyl-3-CH_2); 4.65 (propyl-3-CH_2)/6.82 (phenyl-2-C*H*, phenyl-6-C*H*), 5.39 (propyl-1-CH_2). MS (EI): *m/z* (%) 386 (5) [M$^+$], 181 (100).

5.1.6. 1-[3-(4-Octylphenoxy)-2-oxopropyl]pyrazole-3-carboxylic acid (35)

A solution of **34** (80 mg, 0.21 mmol) in ethanol (9 mL) was treated with a solution of 10% aqueous KOH (3 mL). The mixture was stirred at room temperature for 2 h. After acidification with 3 M HCl, the reaction mixture was extracted exhaustively with CH_2Cl_2/ethyl acetate (3:1). The combined organic layers were washed with water, dried (Na_2SO_4) and the solvent was distilled off. Chromatography on silica gel (CH_2Cl_2/methanol/formic acid, 98:2:0.5) yielded **35** as a solid (5 mg, 6%). ^{1}H NMR (DMSO-d_6): δ 0.84 (t, J = 6.8 Hz, 3H), 1.19–1.29 (m, 10H), 1.47–1.53 (m, 2H), 2.45–2.51 (m, 2H), 4.91 (s, 2H), 5.40 (s, 2H), 6.71–6.73 (m, 1H), 6.85 (d, J = 8.6 Hz, 2H), 7.09 (d, J = 8.4 Hz, 2H), 7.75–7.77 (m, 1H). MS (ESI-): *m/z* 371 [M−H]$^-$.

5.1.7. Ethyl 1-[2-hydroxy-3-(4-octylphenoxy)propyl]pyrazole-4-carboxylate (36)

A mixture of ethyl pyrazole-4-carboxylate (200 mg, 1.4 mmol) and 2-(4-octylphenoxymethyl)oxirane (380 mg, 1.4 mmol) was heated at 80 °C for 4 h. The obtained melt was dissolved in toluene and chromatographed on silica gel (CH_2Cl_2/methanol, 99:1) to yield **36** (373 mg, 65 %) as solid; mp 84 °C. ^{1}H NMR ($CDCl_3$): δ 0.87 (t, J = 7.0 Hz, 3H), 1.26–1.29 (m, 10H), 1.33 (t, J = 7.2 Hz, 3H), 1.53–1.60 (m, 2H), 2.54 (t, J = 7.2 Hz, 2H), 3.50 (s, br, 1H), 3.86 (dd, J = 9.7 Hz and 5.6 Hz, 1H), 3.95 (dd, J = 9.7 Hz and 4.8 Hz, 1H), 4.28 (q, J = 7.2 Hz, 2H), 4.31–4.34 (m, 1H), 4.36–4.41 (m, 1H), 4.44 (dd, J = 12.9 Hz and 2.8 Hz, 1H), 6.80 (dd, J = 8.6 Hz and 2.0 Hz, 2H), 7.09 (dd, J = 8.6 Hz and 2.4 Hz, 2H), 7.94 (s, 1H), 7.97 (s, 1H). MS (EI): *m/z* (%) 402 (4) [M$^+$], 197 (100).

5.1.8. Ethyl 1-[3-(4-octylphenoxy)-2-oxopropyl]pyrazole-4-carboxylate (37)

Compound **36** (167 mg, 0.41 mmol) was oxidized with Dess–Martin periodinane (264 mg, 0.62 mmol) as described above for the synthesis of **29**. The crude product was purified by silica gel chromatography (CH_2Cl_2/methanol, 99:1) to yield **37** as a solid (121 mg, 73%); mp 88 °C. ^{1}H NMR ($CDCl_3$): δ 0.88 (t, J = 7.1 Hz, 3H), 1.27–1.30 (m, 10H), 1.34 (t, J = 7.0 Hz, 3H), 1.54–1.61 (m, 2H), 2.56 (t, J = 7.8 Hz, 2H), 4.30 (q, J = 7.2 Hz, 2H), 4.67 (s, 2H), 5.30 (s, 2H), 6.83 (d, J = 8.6 Hz, 2H), 7.13 (d, J = 8.6 Hz, 2H), 7.93 (s, 1H), 7.96 (s, 1H). MS (EI): *m/z* (%) 400 (16) [M$^+$], 167 (100).

5.1.9. 1-[3-(4-Octylphenoxy)-2-oxopropyl]pyrazole-4-carboxylic acid (38)

Compound **37** (91 mg, 0.23 mmol) was hydrolyzed according to the procedure described above for the preparation of **35**. Chromatography on silica gel (CH_2Cl_2/methanol/formic acid, 99:1:0.5) yielded **38** as a solid (38 mg, 45%); mp 121 °C. ^{1}H NMR ($CDCl_3$): δ 0.87 (t, J = 7.0 Hz, 3H), 1.26–1.33 (m, 10H), 1.55–1.59 (m, 2H), 2.55 (t, J = 7.4 Hz, 2H), 4.68 (s, 2H), 5.33 (s, 2H), 6.83 (d, J = 8.6 Hz, 2H), 7.11 (d, J = 8.6 Hz, 2H), 7.98 (s, 1H), 8.01 (s, 1H). MS (EI): *m/z* (%) 373 (2) [M$^+$], 167 (100).

5.1.10. 1-[3-(4-Octylphenoxy)-2-oxopropyl]pyrazole-5-carboxylic acid (39)

Compound **39** was prepared from **33b** by the procedures described for the preparation of **35**. Chromatography on silica gel (CH_2Cl_2/methanol/formic acid, 98:2:0.5) yielded **39** as a solid; mp 97 °C. ^{1}H NMR (DMSO-d_6): δ 0.83 (t, J = 7.0 Hz, 3H), 1.22–1.24 (m, 10H), 1.49–1.52 (m, 2H), 2.46–2.5 (m, 2H), 4.94 (s, 2H), 5.55 (s, 2H), 6.84 (d, J = 8.6 Hz, 2H), 6.87 (d, J = 2.0 Hz, 1H), 7.07 (d, J = 8.6 Hz, 2H), 7.57 (d, J = 2.0 Hz, 1H). MS (ESI-): *m/z* 371 [M−H]$^-$.

5.1.11. Methyl 1-[2-hydroxy-3-(4-octylphenoxy)propyl]imidazole-4-carboxylate (40a) and -5-carboxylate (40b)

A mixture of methyl imidazole-4-carboxylate (96 mg, 0.76 mmol) and 2-(4-octylphenoxymethyl)oxirane (200 mg, 0.76 mmol) was heated at 80 °C for 5 h. The obtained melt was chromatographed on silica gel (CH_2Cl_2/methanol, 99.5:0.5) to yield **40a** (60 mg, 20%) and **40b** (15 mg, 5 %) as solids.

Compound **40a**: mp 111 °C. ^{1}H NMR ($CDCl_3$): δ 0.88 (t, J = 7.1 Hz, 3H), 1.26–1.29 (m, 10H), 1.55–1.59 (m, 2H), 2.55 (t, J = 7.4 Hz, 2H), 3.86 (s, 3H), 3.92–4.01 (m, 2H), 4.11 (dd, J = 14.0 Hz and 7.6 Hz, 1H), 4.27 (dd, J = 14.0 Hz and 3.4 Hz, 1H), 4.31–4.37 (m, 1H), 6.83 (dd, J = 8.6 Hz and 2.0 Hz, 2H), 7.10 (d, J = 8.6 Hz, 2H), 7.50 (s, 1H), 7.64 (d, J = 1.2 Hz, 1H). MS (EI): *m/z* (%) 389(18) [M$^+$], 303 (100).

Compound **40b**: mp 83 °C. ^{1}H NMR ($CDCl_3$): δ 0.87 (t, J = 7.0 Hz, 3H), 1.26–1.3 (m, 10H), 1.55–1.58 (m, 2H), 2.54 (t, J = 7.4 Hz, 2H), 3.45 (d, J = 5.1 Hz, 1H), 3.84 (s, 3H), 3.95 (dd, J = 9.6 Hz and 5.1 Hz, 1H), 4.0 (dd, J = 9.6 Hz and 4.8 Hz, 1H), 4.25–4.32 (m, 1H), 4.41 (dd, J = 14.1 Hz and 7.5 Hz, 1H), 4.69 (dd, J = 14.1 Hz and 3.3 Hz, 1H), 6.82 (dd, J = 8.6 Hz and 2.0 Hz, 2H), 7.09 (dd, J = 8.6 Hz and 2.0 Hz, 2H), 7.67 (d, J = 0.8 Hz, 1H), 7.72 (d, J = 1.2 Hz, 1H). MS (EI): *m/z* (%) 389 (6) [M$^+$], 107 (100).

5.1.12. 1-[3-(4-Octylphenoxy)-2-oxopropyl]imidazole-4-carboxylic acid (41)

Compound **41** was prepared from **40a** by the procedures described for the preparation of **35**; mp 168 °C. ^{1}H NMR (DMSO-d_6): δ 0.83 (t, J = 6.7 Hz, 3H), 1.22–1.29 (m, 10H), 1.45–1.54 (m, 2H), 2.44–2.52 (m, 2H), 4.88 (s, 2H), 5.25 (s, 2H), 6.87 (d, J = 6.9 Hz, 2H), 7.10 (d, J = 8.2 Hz, 2H), 7.60 (s, 1H), 7.70 (s, br, 1H). MS (ESI-): *m/z* 371 [M−H]$^-$.

5.1.13. 1-[3-(4-Octylphenoxy)-2-oxopropyl]imidazole-5-carboxylic acid (42)

Compound **42** was prepared from **40b** by the procedures described for the preparation of **35**; mp 154 °C. ^{1}H NMR (DMSO-d_6): δ 0.84 (t, J = 6.7 Hz, 3H), 1.22–1.24 (m, 10H), 1.48–1.52 (m, 2H), 2.46–2.50 (m, 2H), 4.91 (s, 2H), 5.33 (s, 2H), 6.86 (d, J = 8.6 Hz, 2H), 7.07 (d, J = 8.6 Hz, 2H), 7.54 (s, br, 1H), 7.79 (s, br, 1H). MS (ESI-): *m/z* 371 [M−H]$^-$.

5.1.14. 1-Benzimidazol-1-yl-3-(4-octylphenoxy)propan-2-one (44)

Compound **44** was synthesized from benzimidazole (54 mg, 0.46 mmol) analogously to the corresponding imidazole derivative **30**. Chromatography on silica gel (CH_2Cl_2/methanol, 98:2) yielded **41** as a solid (20 mg, 12%); mp 101 °C. ^{1}H NMR ($CDCl_3$): δ 0.84 (t, J = 6.8 Hz, 3H), 1.18–1.29 (m, 10H), 1.46–1.54 (m, 2H), 2.45–2.51 (m, 2H), 5.01 (s, 2H), 5.48 (s, 2H), 6.89 (d, J = 8.6 Hz, 2H), 7.11 (d, J = 8.5 Hz, 2H), 7.17–7.23 (m, 2H), 7.46–7.50 (m, 1H), 7.63–7.67 (m, 1H), 8.10 (s, 1H). MS (EI): *m/z* (%) 378 (3) [M$^+$].

5.1.15. 1-Oxiranylmethylbenzotriazole (45)

A mixture of benzotriazole (0.50 g, 4.2 mmol), powdered KOH (88%, 0.48 g, 7.5 mmol), tetrabutylammonium bromide (135 mg, 0.42 mmol) and epichlorohydrin (4 mL) was stirred at room temperature for 4 h. The reaction mixture was purified by silica gel chromatography (hexane/ethyl acetate, 9:1 followed by 8:2) to give **45** as an oil (0.44 g, 60%). ^{1}H NMR ($CDCl_3$): δ 2.62–2.66 (m, 1H), 2.90–2.94 (m, 1H), 3.42–3.46 (m, 1H), 4.59 (dd, J = 15,1 Hz and 6.0 Hz, 1H), 5.10 (dd, J = 15.1 Hz and 3.0 Hz, 1H), 7.37–7.42 (m, 1H), 7.49–7.55 (m, 1H), 7.66–7.70 (m, 1H), 8.05–8.09 (m, 1H). MS (EI): m/z (%) 175 (91) [M^+], 146 (100).

5.1.16. 1-Benzotriazol-1-yl-3-(4-octylphenoxy)propan-2-ol (46)

Compound **45** (0.43 g, 2.5 mmol), 4-octylphenol (0.51 g, 2.5 mmol) and 4-dimethylaminopyridine (61 mg) were mixed thoroughly and heated under stirring at 120 °C for 45 min. The warm reaction mixture was dissolved in toluene and subjected to chromatography on silica gel (hexane/ethyl acetate, 8:2) to give **46** as an oil (0.62 g, 66%). ^{1}H NMR ($CDCl_3$): δ (ppm) = 0.87 (t, J = 6.7 Hz, 3H), 1.20–1.35 (m, 10H), 1.51–1.62 (m, 2H), 2.49–2.58 (m, 2H), 3.02 (s, br, 1H), 3.99 (dd, J = 9.6 Hz and 5.6 Hz, 1H). 4.04 (dd, J = 9.6 Hz and 5.2 Hz, 1H), 4.56–4.65 (m, 1H), 4.84 (dd, J = 14.3 Hz and 6.5 Hz, 1H), 4.94 (dd, J = 14.4 Hz and 4.2 Hz, 1H), 6.81 (d, J = 8.5 Hz, 2H), 7.08 (d, J = 8.5 Hz, 2H), 7.38 (t, 1H, J = 7.6 Hz, 1H), 7.49 (t, J = 7.6 Hz, 1H), 7.65 (d, J = 8.4 Hz, 1H), 8.03 (d, J = 8.4 Hz, 1H). MS (EI): m/z (%) 381 (8) [M^+], 176 (100).

5.1.17. 1-Benzotriazol-1-yl-3-(4-octylphenoxy)propan-2-one (47)

Compound **46** (0.31 g, 0.81 mmol) was oxidized with Dess–Martin periodinane (0.51 g, 1.2 mmol) analogously to the synthesis of **29**. The crude product was purified by silica gel chromatography (hexane/ethyl acetate, 8:2) to yield **47** as a solid (0.28 g, 91 %); mp 102–103 °C. ^{1}H NMR ($CDCl_3$): 0.88 (t, J = 6.9 Hz, 3H), 1.23–1.36 (m, 10H), 1.55–1.64 (m, 2H), 2.55–2.61 (m, 2H), 4.75 (s, 2H), 5.80 (s, 2H), 6.85–6.90 (m, 2H), 7.16 (d, J = 8.7 Hz, 2H), 7.29–7.33 (m, 1H), 7.37–7.43 (m, 1H), 7.47–7.52 (m, 1H), 8.08–8.12 (m, 1H). MS (EI): m/z (%) 379 (28) [M^+], 175 (100).

5.2. Biological evaluation of the target compounds

5.2.1. cPLA$_2\alpha$ inhibition assay

The ability of test compounds to inhibit cPLA$_2\alpha$ isolated from human platelets was performed as described earlier.[40] Briefly, sonicated covesicles consisting of 1-stearoyl-2-arachidonoyl-sn-glycero-3-phosphocholine (200 μM) and 1,2-dioleoyl-sn-glycerol (100 μM) were 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. cPLA$_2\alpha$ activity was determined by measuring 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 sample clean-up by solid phase extraction.

5.2.2. FAAH inhibition assay

Inhibition of FAAH by the test substances was evaluated as previously described.[41] Rat brain microsomes served as enzyme source. The substrate *N*-(2-hydroxyethyl)-4-pyren-1-ylbutanamide (100 μM) was solubilized with Triton X-100 (0.2 %). Enzyme reaction was terminated by addition of a mixture of acetonitrile/methanol including the internal standard 6-pyren-1-ylhexanoic acid. FAAH activity was determined directly without further sample clean-up by measuring the amount of 4-pyren-1-ylbutanoic acid released by the enzyme in absence and presence of a test compound with reversed-phase HPLC and fluorescence-detection (excitation: 340 nm; emission: 380 nm). The only deviation from the published procedure was that the incubation time with the enzyme was 45 min instead of 60 min. This lead to a slight change of the IC_{50} values of the reference compounds **5** and **6**.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2009.11.028.

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