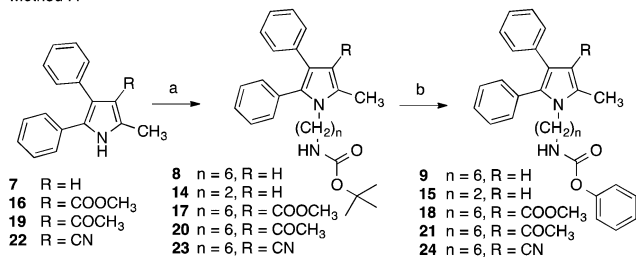
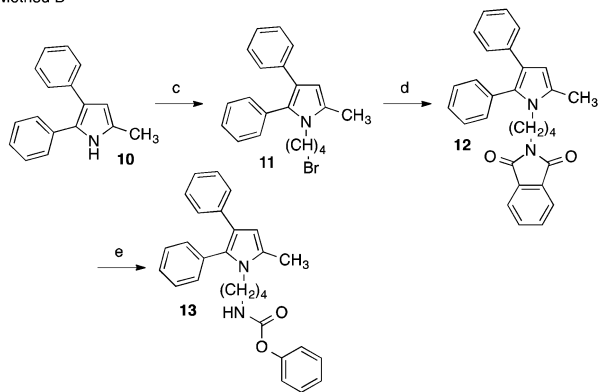


Fig. 2 Structure of the dual cyclooxygenase–5-lipoxygenase inhibitor 6.

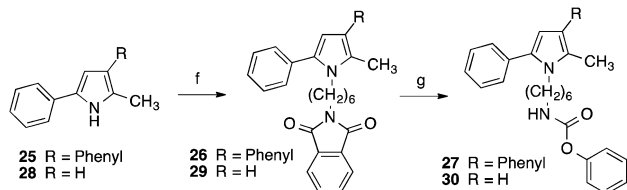
Method A



Method B



Method C



Scheme 1 Reagents and conditions: (a) *tert*-butyl-*N*-(6-bromohexyl)-carbamate–*tert*-butyl-*N*-(2-bromoethyl)carbamate, *K*-*tert*-butylate, DMSO, 60/80 °C; (b) (1) trifluoroacetic acid, CH₂Cl₂, 0 °C; (2) phenyl chloroformate, triethylamine, CH₂Cl₂, room temp.; (c) 1,4-dibromobutane, tetrabutylammonium bromide, toluene, 50% aqueous NaOH, 45 °C; (d) potassium phthalimide, DMF, 130 °C; (e) (1) hydrazine-hydrate, ethanol, reflux; (2) phenyl chloroformate, triethylamine, CH₂Cl₂, room temp.; (f) *N*-(6-bromohexyl)phthalimide, *K*-*tert*-butylate, DMSO, 50/70 °C; (g) (1) hydrazine-hydrate, ethanol, reflux; (2) phenyl chloroformate, triethylamine, CH₂Cl₂, 0 °C to room temp.

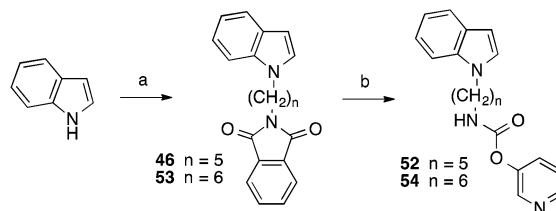
The related derivatives with indole, indazole, imidazole and benzotriazole heterocycle were prepared similarly to Method C of Scheme 1 using *K*-*tert*-butylate–DMSO, NaH–DMSO, NaH–DMF, K₂CO₃–DMSO and K₂CO₃–acetonitrile, respectively, for the *N*-alkylation of the appropriate heterocycle with *N*-(ω -bromoalkyl)phthalimide.²²

The indole derivatives with 3-pyridyl carbamate residues (52 and 54) were synthesized with the latter method utilizing 3-pyridyl chloroformate, synthesized from diphosgene and pyridin-3-ol, instead of phenyl chloroformate in the last step (Scheme 2).

Inhibition of FAAH hydrolase by the test compounds was determined with an assay using microsomes from rat brain as enzyme source and *N*-(2-hydroxyethyl)-4-pyren-1-ylbutanamide as fluorogenic substrate.²³ Inhibitory potencies of the tested compounds were assessed by comparing the amount of 4-pyren-1-ylbutanoic acid released from the substrate in their absence and presence after an incubation time of 60 min by reversed-phase HPLC with fluorescence detection (see ESI†). With this assay for the diphenylimidazole 2 an IC₅₀ of 0.35 μ M was measured (Table 1). Replacement of the imidazole scaffold of 2 by a pyrrole (9) led to an about sevenfold loss of activity. Shortening of the alkyl chain connecting the carbamate group and the pyrrole heterocycle from six to four and two carbon atoms further decreased inhibitory potency. With an IC₅₀ of 6.2 μ M the butyl derivative 13 was only about half as active as the hexyl compound 9. The derivative with an ethyl linker (15) even was inactive at the highest test concentration of 10 μ M. Because removing of the methyl group in position 2 of the imidazole ring of 2 did not change the activity of the compound,²⁴ the impact of the corresponding methyl group of the pyrrole 9 on inhibitory potency was not examined.

Introduction of polar ethoxycarbonyl, acetyl and cyano substituents at the free position of the pyrrole ring of 9 was also detrimental. The inhibition values of obtained compounds 18, 21 and 24 were in the magnitude of 10 μ M (Table 2). Interestingly, moving the phenyl group from position 3 to position 4 of the pyrrole ring, to give compound 27, as well as completely removing the phenyl ring, to give compound 30, significantly increased FAAH inhibition. The activity of these two inhibitors was comparable to that of the diphenylimidazole 2.

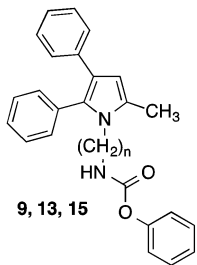
Similar results were obtained with analogous indole derivatives (Table 3). With IC₅₀ values of about 5 μ M the 2,3-diphenylindole 32 and the 3-phenylindole 34 possessed the lowest potency of the series. The 2-phenylindole 36 was about tenfold more active than the 3-phenylindole 34. Thus, a phenyl substituent is much better tolerated in position 2 than in position 3 of the indole scaffold. The indole derivative 38, which has no phenyl-substituents in 2- and 3-position, possessed the highest activity (IC₅₀ 0.25 μ M) showing that phenyl substituents are even unfavourable.



Scheme 2 Reagents and conditions: (a) *N*-(ω -bromoalkyl)phthalimide, NaH, DMSO, 100 °C; (b) (1) hydrazine-hydrate, ethanol, reflux, 2 h; (2) trichloromethyl chloroformate, pyridin-3-ol, ethyl(diisopropyl)amine, CH₂Cl₂, THF, – 30 °C to room temp.



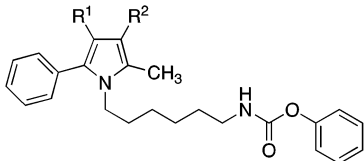
Table 1 Inhibition of FAAH



Compound	<i>n</i>	Inhibition of FAAH IC ₅₀ ^a (μM)
9	6	2.6
13	4	6.2
15	2	n.a. ^b
1 (URB 597)		0.060
2		0.35
3 (PHOP)		0.0029

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

Table 2 Inhibition of FAAH



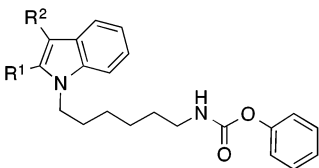
Compound	R ¹	R ²	Inhibition of FAAH IC ₅₀ ^a (μM)
9	Phenyl	H	2.6
18	Phenyl	COOCH ₃	>10 ^b
21	Phenyl	COCH ₃	>10 ^c
24	Phenyl	CN	8.7
27	H	Phenyl	0.46
30	H	H	0.31

^a Values are the means of at least two independent determinations, errors are within ± 20%. ^b 21% inhibition at 10 μM. ^c 33% inhibition at 10 μM.

Next the effect of an introduction of nitrogen atoms in position 2 and 3 of the indole ring of **38** was studied. The indazole and benzotriazole derivatives **40** and **42** bearing a nitrogen in position 2 of the heterocycle were about three- to fourfold less active than the indole **38** (Table 4). In contrast, the imidazole derivative **44** possessing an additional nitrogen only in position 3 was equipotent to the indole **38**.

Taken together, replacement of the diphenylpyrrole heterocycle from the starting compound **9** by an unsubstituted indole (**38**) led to an about tenfold increase of FAAH inhibitory potency. At this point we wanted to evaluate the role of the alkyl linker of **38** on activity in more detail. Both shortening and elongation of the hexyl chain by one carbon increased activity about twofold: the IC₅₀ values of the pentyl and heptyl derivatives **47** and **49**

Table 3 Inhibition of FAAH



Compound	R ¹	R ²	Inhibition of FAAH IC ₅₀ ^a (μM)
32	Phenyl	Phenyl	4.7
34	H	Phenyl	4.9
36	Phenyl	H	0.52
38	H	H	0.25

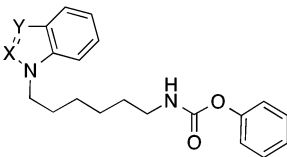
^a Values are the means of at least two independent determinations, errors are within ± 20%.

were about 0.09 μM (Table 5). A more pronounced variation of the chain length to four (**45**) or eight (**51**) carbons resulted in a reduction of FAAH inhibition.

In literature a series of highly potent 3-pyridyl carbamate inhibitors of FAAH is described.²⁵ Therefore, we finally wanted to investigate the effect of a replacement of the phenyl group of the carbamates **47** and **38** by a 3-pyridyl residue. This structural variation led to a drastic increase of activity (Table 5). With IC₅₀ values of 0.0036 μM and 0.0052 μM obtained compounds **52** and **54** were about as active as the α-ketoheterocycle **3** (PHOP), which counts to the most active inhibitors of FAAH known today.

Besides FAAH, monoacylglycerol lipase (MAGL) is another important enzyme during the process of endocannabinoid inactivation. Just like FAAH, MAGL contains a catalytic serine in the active site. The main substrate of MAGL is 2-arachidonoyl glycerol. To get some information about the specificity of the developed substance class, some of the new FAAH inhibitors were also tested for MAGL inhibition.²⁶ As shown in Table 5, the evaluated phenyl ω-(indol-1-yl)alkylcarbamates did not inhibit MAGL at the highest test concentration of 10 μM. Contrary, with

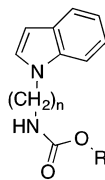
Table 4 Inhibition of FAAH



Compound	X	Y	Inhibition of FAAH IC ₅₀ ^a (μM)
38	CH	CH	0.25
40	N	CH	0.92
42	N	N	0.81
44	CH	N	0.29

^a Values are the means of at least two independent determinations, errors are within ± 20%.



Table 5 Inhibitory potency against FAAH and MAGL and stability in biological environments of several ω -indolylalkylcarbamates

Compound	<i>n</i>	R	Inhibition of FAAH IC ₅₀ ^a (μM)	Inhibition of MAGL IC ₅₀ ^a (μM)	Stability in liver S9 fractions ^b (%)	Stability in blood plasma ^c (%)
45	4	Phenyl	0.96	n.a. ^d	53 ± 4.7	82 ± 9.0
47	5	Phenyl	0.094	n.a. ^d	52 ± 2.3	91 ± 6.5
38	6	Phenyl	0.25	n.a. ^d	50 ± 1.5	68 ± 7.8
49	7	Phenyl	0.090	n.a. ^d	59 ± 3.1	39 ± 7.4
51	8	Phenyl	0.24	n.a. ^d	49 ± 0.3	64 ± 4.2
52	5	Pyridin-3-yl	0.0036	0.22	43 ± 7.0	0
54	6	Pyridin-3-yl	0.0052	0.46	47 ± 13	0
1 (URB 597)			0.060	n.t. ^e	13 ± 4.6	76 ± 12

^a Values are the means of at least two independent determinations, errors are within ± 20%. ^b Percent of parent remaining after incubation with rat liver S9 fractions for 30 min, in presence of the co-factor NADPH; values are means ± standard deviations of independent determinations (*n* = 3). ^c Percent of parent remaining after incubation with porcine blood plasma for 30 min; values are means ± standard deviations of independent determinations (*n* = 3). ^d n.a.: not active at 10 μM. ^e n.t.: not tested.

IC₅₀ values in the submicromolar range, the corresponding pyridyl carbamates **52** and **54** inhibited MAGL significantly. However, the inhibitory potency of these substances against FAAH is still 60- to 90-fold higher than against MAGL.

Another enzyme of the serine hydrolase family is cytosolic phospholipase A₂α (cPLA₂α), which catalyzes the first step of the so called arachidonic acid cascade by cleaving membrane phospholipids in arachidonic acid and lysophospholipids. At the highest test concentration of 10 μM cPLA₂α was not inhibited²⁷ by the indole compounds listed in Table 5. These results indicate that the inhibitors investigated display a certain specificity with regard to their ability to inhibit serine hydrolase enzymes.

Since carbamates are known to be susceptible to hydrolysis in biological environments^{28–30} leading to the inactivation of the compounds, we also measured the stability of some of the new substances in rat liver S9 fractions in presence of the co-factor NADPH³¹ and in porcine blood plasma (Table 5). After incubation in the liver S9 fractions for 30 min, still about 50% of the selected indole compounds were present in the incubation mixture. For comparison, the metabolism rate of the known FAAH inhibitor URB 597, which also exhibits a carbamate structure, was significantly higher. Here, only about 13% of the parent compound could be detected after the metabolic reactions. Incubation in blood plasma for 30 min revealed striking different results. The pyridin-3-yloxy carbamates **52** and **54** had totally disappeared at the end of the trial most likely due to an extensive hydrolysis or transcarbamoylation. The stability of the phenoxycarbamates depended on the chain length of the alkyl spacer. The pentyl-substituted compound **47** exhibited high stability, with 91% parent remaining after incubation. In contrast, only 39% of the heptyl derivative **49** could be detected under the same conditions.

Conclusions

In conclusion, starting from the lead compounds **2** and **9**, respectively, a pronounced increase of FAAH inhibitory potency could be attained by only a few structural variations. The most active compounds synthesized (**52** and **54**) exhibited IC₅₀ values in the low nanomolar range.

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