Hexafluoroisopropyl Carbamates as Selective MAGL and Dual MAGL/FAAH Inhibitors: Biochemical and Physicochemical Properties

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A series of hexafluoroisopropyl carbamates with indolylalkyl- and azaindolylalkyl-substituents at the carbamate nitrogen was synthesized and evaluated for inhibition of the endocannabinoid degrading enzymes fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL). The synthesized derivatives with butyl to heptyl spacers between the heteroaryl and the carbamate moiety were inhibitors of both enzymes. For investigated compounds in which the alkyl chain was partially incorporated into a piperidine ring, different results were obtained. Compounds with a methylene spacer between the piperidine ring and the heteroaromatic system were found to be selective MAGL inhibitors, while an extension of the alkyl spacer to two to four atoms resulted in dual inhibition of FAAH/MAGL. The only small change in enzyme inhibitory activity with variation of the heteroaromatic system indicates that the reactive hexafluoroisopropyl carbamate group is mainly responsible for the strength of the inhibitory effect of the compounds. Selected derivatives were also tested for hydrolytic stability in aqueous solution, liver homogenate and blood plasma as well as for aqueous solubility and for permeability in a Caco-2 cell model. Some compounds showed a slightly higher MAGL inhibitory effect than the known selective MAGL inhibitor ABX-1431 and also partly surpassed this substance with regard to certain physicochemical and biochemical properties such as water solubility and cell permeability.

Introduction

In the 1980s, the American President Ronald Reagan released several million dollars for studies, which should prove that marijuana damages the human brain. With this money, a series of investigations were subsided that led to the discovery of the endocannabinoid system.[1,2] This biological pathway, which is widely distributed throughout the organism, consists of the endocannabinoid receptors CB1 and CB2, their natural ligands, the endocannabinoids, and the enzymes, responsible for endocannabinoid degradation.[3–6] The two most prominent endocannabinoids are arachidonoyl ethanolamide, named as anandamide, and 2-arachidonoyl glycerol (2-AG). They are produced from phospholipids „on demand" and mediate diverse pharmacological effects. For their inactivation the two serine hydrolases fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL) are responsible. While the amide anandamide is primarily degraded by FAAH, the ester compound 2-AG can be cleaved by both enzymes in principal.

Genetic or pharmacological disruption of FAAH or MAGL resulted in an increase in endocannabinoid levels in the nervous system that was associated with antihyperalgesia and antianxiety in multiple *in vivo* assays.[7] These outcomes stimulated the search for inhibitors of the endocannabinoid-degrading enzymes. Since a prolonged inhibition of MAGL in animals caused a loss of analgesic response to the inhibitor and a down regulation of CB1 receptors,[8] which was not seen following FAAH blockade, the focus was initially directed on the development of FAAH inhibitors.[9,10] These agents, which in particular include carbamates, like URB597 (**1**) (Figure 1), urea derivatives and activated ketones, showed promising effects during their preclinical development in various animal models. In phase II studies on humans, however, the substances could not fulfil the expectations placed in them. The urea derivative PF-04457845 (**2**), developed by Pfizer, did not produce analgesic effects in patients with osteoarthritis of the knee.[11] Vernalis' FAAH inhibitor V158866 also failed to show the expected effects in a phase II study for the treatment of neuropathic pain resulting from spinal cord injury.[12] In addition, Sanofi's FAAH inhibitor SSR411298 (**3**) was no more effective than placebo in a phase II trial for the treatment of major depressive disorder in elderly patients.[13] For these reasons, among others, the focus of research shifted to inhibitors of MAGL.[14–16] Thus, MAGL not only controls the 2-AG level in the brain, but is also significantly involved in the formation of the pro-inflammatory eicosanoid

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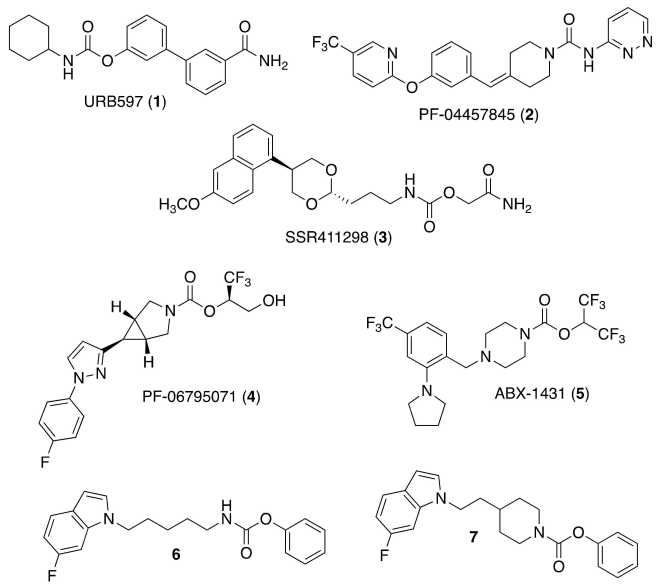


Figure 1. Structures of known FAAH and MAGL inhibitors.

precursor arachidonic acid.[17] While in the periphery arachidonic acid is primarily formed from phospholipids via cytosolic phospholipase $A_2\alpha$ (cPLA$_2\alpha$), in the brain this fatty acid mainly originates from 2-AG cleavage.[18] Since the conversion of arachidonic acid by cyclooxygenase and lipoxygenase enzymes leads to the formation of inflammatory prostaglandins and leukotrienes, MAGL inhibitors may have therapeutic potential in the treatment of neuroinflammation, among other things. In recent years, a number of inhibitors for MAGL have been published, including the urea derivative JJKK048[19] and carbamates[20,21] such as PF-06795071 (**4**)[18] and ABX-1431 (**5**) (Figure 1).[22] The latter compound is currently under evaluation in human clinical trials.

Recently, we have developed phenyl *N*-[ω-(6-fluoroindol-1-yl)alkyl]carbamates and analogous 4-alkylpiperidine-1-carboxylates with high FAAH inhibitory potency, such as compounds **6**[23] and **7**.[24] The activity of MAGL was not affected by these substances. In an attempt to create compounds that act as selective MAGL or dual MAGL/FAAH inhibitors, we have now replaced the phenyl residue of the carbamate moiety of these agents with a 1,1,1,3,3,3-hexafluoropropan-2-yl group present in several selective carbamate-based covalently binding MAGL inhibitors.[20–22] These compounds attack the serine in the active site of MAGL via their carbamate carbonyl moiety, which leads to carbamoylation of the enzyme with the exit of the hexafluoroisopropanol leaving group. Our structure-activity relationship studies included variation in the length of the alkyl spacer of the new carbamates and the substitution pattern of the indole heterocycle. Selected compounds were tested for hydrolytic stability in phosphate buffer, liver homogenate, and blood plasma. In addition, to obtain information on the possibility of absorption after oral administration, we determined the aqueous solubility as well as the permeability of some of these compounds in a Caco-2 cell culture model.

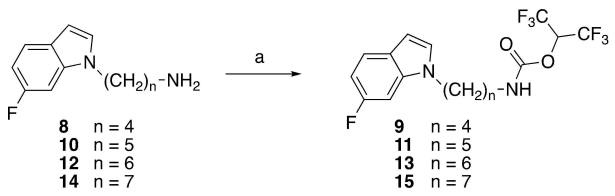
Results and Discussion

Chemical Synthesis

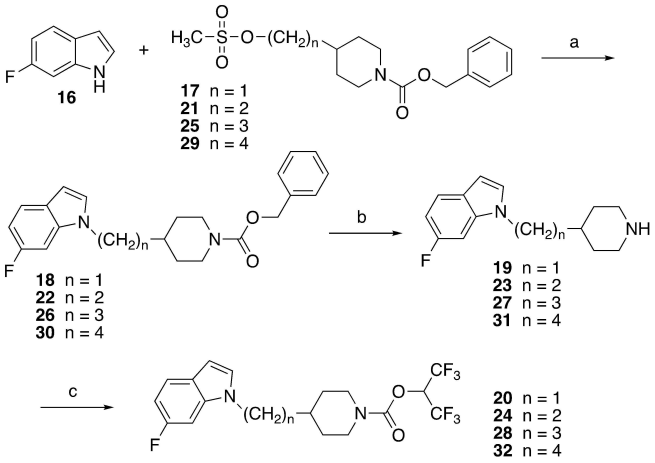
The synthesis of the 6-fluoroindole-substituted target compounds with alkyl spacer between carbamate group and indole heterocycle (**9**, **11**, **13**, **15**) were obtained by reaction of the appropriate indolyl-substituted alkan-1-amines (**8**, **10**, **12**, **14**)[23,24] with an activated carbonate formed from 1,1,1,3,3,3-hexafluoropropan-2-ol and bis(pentafluorophenyl) carbonate in acetonitrile in presence of triethylamine (Scheme 1).[18]

For the synthesis of the indol-1-ylalkyl-substituted piperidine carbamates **20**, **24**, **28** and **32**, 6-fluoroindole was *N*-alkylated with the corresponding 4-(mesyloxyalkyl)piperidine protected as its benzyloxycarbonyl derivative (**17**, **21**, **25**, **29**)[25] using sodium hydride as base (Scheme 2). The protecting group of the formed intermediates **18**, **22**, **26** and **30** was removed by catalytic hydrogenation and the resulting free secondary amine (**19**, **23**, **27**, **31**) acylated with an activated 1,1,1,3,3,3-hexafluoropropyl carbonate as described above to obtain the desired carbamates.

The synthesis of the derivative of compound **11** methylated at the carbamate nitrogen was carried out starting from 5-(6-



Scheme 1. Reagents and conditions: (a) 1,1,1,3,3,3-Hexafluoropropan-2-ol, bis(pentafluorophenyl) carbonate, triethylamine, acetonitrile, 0 °C for 30 min followed by room temperature for 2 h; addition of **8**, **10**, **12** or **14**, triethylamine, acetonitrile, room temperature, overnight.



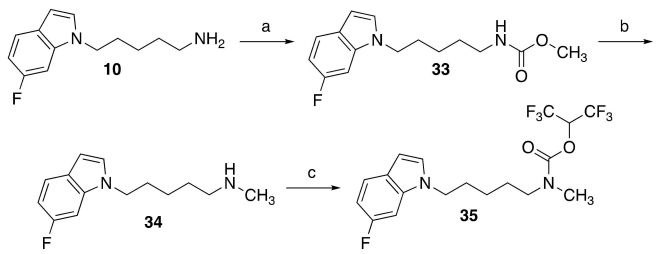
Scheme 2. Reagents and conditions: (a) Sodium hydride, DMF, room temperature, overnight; (b) H_2, Pd/C, methanol/THF (1 : 1), room temperature, 4 h; (c) 1,1,1,3,3,3-hexafluoropropan-2-ol, bis(pentafluorophenyl) carbonate, triethylamine, acetonitrile, 0 °C for 30 min followed by room temperature for 2 h; addition of **19**, **23**, **27** or **31**, triethylamine, acetonitrile, room temperature, overnight.

fluoroindol-1-yl)pentan-1-amine (**10**) (Scheme 3). The reaction with methyl chloroformate and subsequent reduction of the carbamate formed (**33**)[26] led to the secondary amine **34**, which was converted to the hexafluoroisopropyl carbamate **35** as described above.

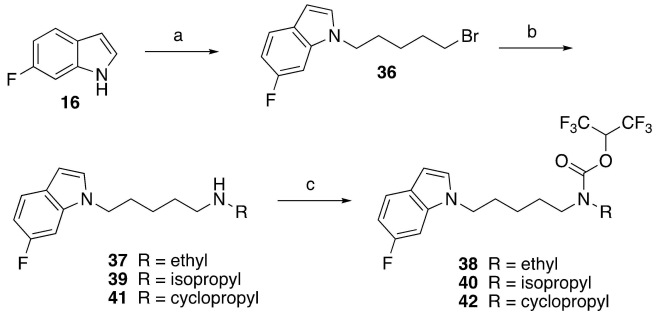
To prepare the corresponding *N*-ethyl, *N*-isopropyl and *N*-cyclopropyl derivatives **38**, **40** and **42**, 6-fluoroindole was first treated with an excess of 1,5-dibromopentane after deprotonation with sodium hydride in DMF (Scheme 4). Substitution of the terminal bromine atom of the formed compound **36** by the appropriate amine residue was achieved by reaction with the corresponding primary amine in DMF in presence of cesium carbonate.[27] Finally, the hexafluoroisopropyl carbamate moiety was introduced by the method outlined above.

Derivatives of **20** and **24**, in which the 6-fluoroindole heterocycle was replaced by an unsubstituted indole, a differently substituted indole or an azaindole residue were obtained in the same manner as these lead compounds using the corresponding heterocycle as starting material. Only the targeted 5-chloroindole-substituted compounds could not be obtained in this way because during the hydrogenolytic cleavage of the benzyloxycarbonyl protecting group, the chlorine atom in the indole ring was simultaneously replaced by a hydrogen atom. However, the desired chlorine-substituted compound with ethyl spacer between 5-chloroindole and piperidine ring system (**78**) could be prepared by alternative cleavage of the benzyloxycarbonyl group with HBr in acetic acid[28] (Scheme 5). In contrast to the synthesis of the other hexafluoroisopropyl carbamates, the subsequent conversion of the piperidine group into the carbamate was carried out by reaction with an activated carbamate produced from 1,1,1,3,3,3-hexafluoropropan-2-ol and triphosgene in acetonitrile in presence of triethylamine.

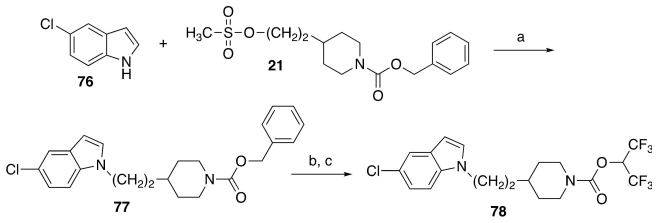
The synthesis of the derivative of **24**, which contained a piperazine instead of a piperidine ring was synthesized as outlined in Scheme 6. 6-Fluoro-1-tosylindole (**88**)[29] was treated with benzyl 4-(2-hydroxyethyl)piperazine-1-carboxylate (**89**) in DMF in the presence of the base potassium *tert*-butylate.[30] Under transfer of the sulfonyl group from the indole to the alcohol a reactive tosylate was provided, which readily alkylated the indole anion in the same step.[31] Cleavage of the benzyloxycarbonyl protecting group by catalytic hydrogenation followed by acylating with an activated 1,1,1,3,3,3-hexafluoropropyl carbonate yielded the target compound **92**.



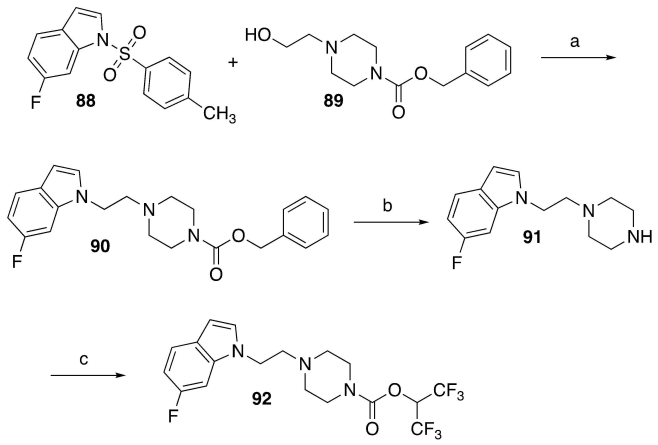
Scheme 3. Reagents and conditions: (a) Methyl chloroformate, triethylamine, 0 °C for 30 min followed by room temperature for 2 h; (b) lithium aluminium hydride, THF, room temperature for 3 h followed by reflux for 2 h; (c) 1,1,1,3,3,3-hexafluoropropan-2-ol, bis(pentafluorophenyl) carbonate, triethylamine, acetonitrile, 0 °C for 30 min followed by room temperature for 2 h; addition of **34**, triethylamine, acetonitrile, room temperature, overnight.



Scheme 4. Reagents and conditions: (a) Sodium hydride, 1,5-dibromopentane, DMF, 90 °C, 3 h (b) alkylamine, cesium carbonate, DMF, room temperature, 24 h; (c) 1,1,1,3,3,3-hexafluoropropan-2-ol, bis(pentafluorophenyl) carbonate, triethylamine, acetonitrile, 0 °C for 30 min followed by room temperature for 2 h; addition of **37**, **39** or **41**, triethylamine, acetonitrile, room temperature, overnight.



Scheme 5. Reagents and conditions: (a) Sodium hydride, DMF, room temperature, overnight; (b) HBr (30 %) in acetic acid, room temperature, 1.5 h; (c) 1,1,1,3,3,3-hexafluoropropan-2-ol, triphosgene, triethylamine, acetonitrile, 0 °C for 30 min followed by room temperature for 2 h; addition of 5-chloro-1-[2-(piperidin-4-yl)ethyl]indole (product of step b), triethylamine, acetonitrile, room temperature, overnight.



Scheme 6. Reagents and conditions: (a) Potassium *tert*-butylate, DMF, 90 °C, 24 h; (b) H_2, Pd/C, methanol/THF (1 : 1), room temperature, 4 h; (c) 1,1,1,3,3,3-hexafluoropropan-2-ol, bis(pentafluorophenyl) carbonate, triethylamine, acetonitrile, 0 °C for 30 min followed by room temperature for 2 h; addition of **91**, triethylamine, acetonitrile, room temperature, overnight.

Biochemical and Physicochemical Evaluation

The inhibitory potency of the target compounds towards FAAH was determined by measuring the release of 4-pyren-1-ylbutanoic acid from the fluorogenic substrate *N*-(2-hydroxyethyl)-4-pyren-1-ylbutanamide with HPLC and fluorescence detection using rat brain homogenate as the enzyme source.[24,32] Inhibition of MAGL was accordingly assessed by quantifying the 4-pyren-1-ylbutanoic acid liberated from the fluorogenic substrate 1,3-dihydroxypropan-2-yl 4-pyren-1-ylbutanoate by human recombinant MAGL.[33]

With these assays, nanomolar IC_{50} values for FAAH inhibition were measured for the phenyl *N*-[5-(6-fluoroindol-1-yl)pentyl]-carbamate (**6**) and the structurally related 6-fluoroindole-substituted 4-ethylpiperidine-1-carboxylate **7** (Table 1), while no inhibition of MAGL by these compounds was detected even at the highest assay concentration of 10 μM. Replacement of the phenyl residue of carbamate **6** by a hexafluoroisopropyl moiety, which is also present in the potent selective MAGL inhibitor ABX-1431 (Figure 1),[22] reduced the inhibitory potency against FAAH by 25-fold. On the other hand, this structural variation resulted in some inhibition of MAGL, as indicated by the IC_{50} value against this enzyme of 3.2 μM. Shortening the alkyl chain by one carbon atom caused an approximately threefold decrease of the inhibitory activity towards both FAAH and MAGL, whereas lengthening by one carbon atom slightly enhanced activity against MAGL without affecting activity towards FAAH. Extension by two carbon atoms also had no significant effect on FAAH inhibition, and the IC_{50} value remained at about 1 μM. In contrast, MAGL inhibitory potency was reduced by about 3–4 fold.

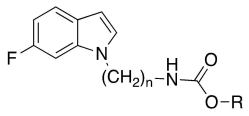
Unlike the phenyl 4-ethylpiperidine-1-carboxylate **7**, the corresponding hexafluoroisopropyl derivative **24** exhibited a significant MAGL inhibitory potency. With an IC_{50} of 0.51 μM, **24** possessed a similar activity against this enzyme as the reference ABX-1431 (IC_{50}: 0.58 μM). The IC_{50} of 1 μM towards FAAH shows the compound to be a well balanced dual inhibitor of the two enzymes. The shortening of the ethylene spacer by one carbon atom was accompanied by a loss of FAAH inhibitory activity, while MAGL activity was retained. Thus, like ABX-1431, the obtained compound **20** is a selective inhibitor of MAGL. Lengthening the ethylene spacer of **24** by one carbon atom (**28**) increased inhibition strength against MAGL about twofold, while introduction of a further carbon (**32**) reduced the effect again to the same extent. With regard to FAAH inhibition, this chain variation led first to a decrease and then again to an increase in potency. The same ups and downs of IC_{50} values for FAAH inhibition upon extension of the ethylene spacer had also been observed for the analogous phenyl indolylethylpiperidine-1-carboxyates.[23] In general, it is noticeable that the alkyl carbamates **9**, **11**, **13** and **15** inhibit FAAH more strongly than MAGL, while conversely the piperidine carboxylates **20**, **24**, **28** and **32** have a higher activity towards MAGL than towards FAAH with regard to their IC_{50} values.

The methylation of the carbamate nitrogen led to a considerable loss of inhibitory activity against FAAH and MAGL. Thus, at the highest test concentration of 10 μM, both enzymes were only inhibited to about 30% by the *N*-methylated derivative **35** (Scheme 3). With the introduction of an ethyl group on this nitrogen (**38**), which can also be seen as cutting the piperidine ring of **24** between carbon 3 and 4, and with substitution by isopropyl (**40**) and cyclopropyl (**42**), compounds were obtained (Scheme 4) that were even inactive at 10 μM.

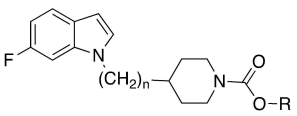
For further structure-activity relationship studies, the selective potent MAGL inhibitor **20** and the well-balanced dual FAAH/MAGL inhibitor **24** were selected. Derivatives of these compounds without fluorine atom on the indole ring were synthesized. In addition, the position of this atom in the indole ring was varied and derivatives with other substituents at position 5 of the indole were investigated.

Interestingly, in the case of the selective MAGL inhibitor **20**, the MAGL inhibitory effect changed only slightly as a result of

Table 1. Inhibitory potency against FAAH and MAGL of various 6-fluoroindole-substituted *N*-alkylcarbamates and piperidine-1-carboxylates.



6, **9**, **11**, **13**, **15**



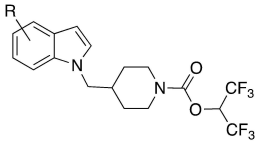
7, **20**, **24**, **28**, **32**

Comp.	n	R	MAGL IC_{50} [μM][a]	FAAH IC_{50} [μM][a]	Log P[b]
6	5	Phenyl	n.a.	0.029	4.1
9	4	$CH(CF_3)_2$	10	2.6	4.7
11	5	$CH(CF_3)_2$	3.2	0.74	5.1
13	6	$CH(CF_3)_2$	2.0	0.77	5.6
15	7	$CH(CF_3)_2$	8.3	1.0	6.1
7	2	Phenyl	n.a.	0.023	5.0
20	1	$CH(CF_3)_2$	0.55	n.a.	5.6
24	2	$CH(CF_3)_2$	0.51	1.0	6.0
28	3	$CH(CF_3)_2$	0.23	6.3	6.5
32	4	$CH(CF_3)_2$	0.45	2.8	7.0
URB597 (**1**)			n.a.	0.043	
ABX-1431 (**5**)			0.58	n.a.	7.4

[a] IC_{50} values are the means of at least two independent determinations, errors are within ±20%; n.a.: not active at 10 μM.
[b] Evaluated by reversed-phase HPLC with acetonitrile/phosphate buffered saline (pH 7.4) as mobile phase.

these structural variations. The IC_{50} values of the derivatives differently substituted at the indole ring against the enzyme only ranged from 0.4 μM to 0.9 μM (Table 2). For the derivatives with a fluorine atom in position 4 (**48**), 5 (**51**) or 7 (**54**) of the indole ring as well as for the corresponding unsubstituted compound **45**, no inhibitory activity towards FAAH was measured at 10 μM as for the lead structure **20**. The derivatives **57**, **60** and **63** with a methyl, methoxy or cyano group in position 5 of the indole ring showed a very weak FAAH inhibition of about 25% at this test concentration.

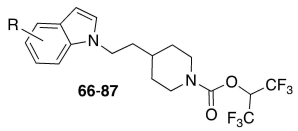
Table 2. Inhibitory potency against FAAH and MAGL of 4-(indol-1-ylmethyl)piperidine-1-carboxylates differently substituted on the indole ring.



Comp.	R	MAGL IC_{50} [μM][a]	FAAH IC_{50} [μM][a]	Log P[b]
45	H	0.55	n.a.	5.5
48	4-F	0.50	n.a.	5.6
51	5-F	0.83	n.a.	5.5
20	6-F	0.55	n.a.	5.6
54	7-F	0.58	n.a.	5.9
57	5-CH_3	0.90	>10[c]	6.1
60	5-OCH_3	0.66	>10[d]	5.2
63	5-CN	0.41	>10[e]	4.7

[a] IC_{50} values are the means of at least two independent determinations, errors are within ±20%.
[b] Evaluated by reversed-phase HPLC with acetonitrile/phosphate buffered saline (pH 7.4) as mobile phase
[c] 26% inhibition at 10 μM (n = 3).
[d] 24% inhibition at 10 μM (n = 3).
[e] 28% inhibition at 10 μM (n = 3).

Table 3. Inhibitory potency against FAAH and MAGL of 4-(indol-1-ylethyl)-piperidine-1-carboxylates differently substituted on the indole ring and of a piperazine-1-carboxylate.



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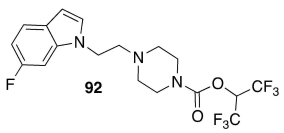


92

Comp.	R	MAGL IC_{50}[μM][a]	FAAH IC_{50} [μM][a]	Log P[b]
66	H	0.62	2.1	5.9
69	4-F	0.47	2.5	6.0
72	5-F	0.38	2.8	5.9
24	6-F	0.51	1.0	6.0
75	7-F	0.46	1.2	6.3
78	5-Cl	0.31	7.5	6.6
81	5-CH_3	0.85	>10[c]	6.5
84	5-OCH_3	0.51	8.3	5.6
87	5-CN	0.70	>10[d]	5.1
92		0.83	2.6	5.0

[a] IC_{50} values are the means of at least two independent determinations, errors are within ±20%.
[b] Evaluated by reversed-phase HPLC with acetonitrile/phosphate buffered saline (pH 7.4) as mobile phase.
[c] 36% inhibition at 10 μM (n = 2).
[d] 45% inhibition at 10 μM (n = 2).

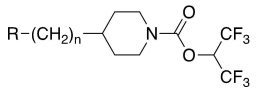
In case of the dual MAGL/FAAH inhibitor **24**, the corresponding structural variations also did not lead to a significant change in the inhibition values against MAGL (Table 3). Again, the IC_{50} values of the compounds evaluated were in approximately the same range (0.3 μM to 0.9 μM). In contrast, the position or nature of the substituents on the indole ring had a slightly greater effect on the inhibitory activity towards FAAH. While the 7-fluoro derivative **75** was about as effective as the lead structure **24**, moving the fluorine atom to positions 4 (**69**) or 5 (**72**) reduced potency by a factor of 2–3. Replacing the 5-fluoro atom of **72** with chlorine (**75**), methyl (**81**), methoxy (**84**) and cyano (**87**) moieties resulted in a further loss of inhibitory efficacy.

In the potent MAGL inhibitor ABX-1431,[22] which was used as a reference in these studies, the reactive carbamate moiety is part of a piperazine residue, whereas in the compounds we synthesized it is part of a piperidine heterocycle. We were therefore interested in the effect of replacing the piperidine with a piperazine ring in the lead structure **24** in terms of inhibition of MAGL or FAAH. As the IC_{50} values of the corresponding piperazine derivative **92** showed, the activity towards the two enzymes decreases by a factor of about 1.5 and 2.5, respectively, due to this structural variation. The undesirably high lipophilicity of the substance, on the other hand, was significantly reduced by this modification, which is reflected in the decrease in the log P value from 6 to 5.

Another way to reduce the undesirably high lipophilicity of lead structure **24** was to introduce nitrogen atoms into the indole heterocycle. We therefore prepared and studied different derivatives of this compound with azaindole ring systems. It was found that the strength of MAGL inhibition was not significantly affected by these variations (Table 4). However, the range of IC_{50} values measured, from 0.15 μM to 1 μM, was slightly larger than that obtained with the compounds prepared before. The IC_{50} values against FAAH varied from 1 μM to slightly more than 10 μM. Among the best dual MAGL/FAAH inhibitors in this series were the benzimidazole **101** and the 7-azaindole **119**, so derivatives of these two compounds were finally prepared with the ethylene spacer replaced by a methylene spacer to obtain potent selective MAGL inhibitors. As expected, both compounds (**122** and **125**) were ineffective against FAAH. However, in the case of the benzimidazole, the MAGL inhibitory potency decreased by about a factor of 4 with the shortening of the ethylene spacer by one carbon atom. In contrast, the 7-azaindole **125** with methylene spacer was about twice as effective against this enzyme as the parent compound **119**. With an IC_{50} of 0.18 μM, **125** was among the most potent of the MAGL inhibitors prepared in this study and also exceeded the activity of the reference compound ABX-1431. As expected, the log P values of all azaindoles were lower than those of the corresponding indole or fluoroindole derivatives. The most polar compound in this series was the benzimidazole-substituted derivative **122** with a log P of 2.9.

Depending on the substitution pattern, carbamates are basically more or less sensitive to hydrolysis. To investigate the chemical and metabolic stability of the synthesized carbamates, we tested the hydrolytic degradation of selected derivatives in

Table 4. Inhibitory potency against FAAH and MAGL of 4-ethyl- and 4-methylpiperidine-1-carboxylates with different azaindole-substituents.



Comp.	n	R	MAGL IC_{50} [μM][a]	FAAH IC_{50} [μM][a]	Log P[b]
66	2	Indol-1-yl	0.64	2.1	5.9
95	2	Indazol-1-yl	0.33	4.1	4.9
98	2	Indazol-2-yl	0.17	>10[c]	4.1
101	2	Benzimidazol-1-yl	0.15	1.0	3.4
104	2	Benzotriazol-1-yl	0.19	>10[d]	3.9
107	2	Benzotriazol-2-yl	0.18	>10[e]	4.8
110	2	4-Azaindol-1-yl	0.30	6.1	3.4
113	2	5-Azaindol-1-yl	1.1	>10[f]	3.4
116	2	6-Azaindol-1-yl	0.56	4.1	3.5
119	2	7-Azaindol-1-yl	0.35	3.9	4.8
45	1	Indol-1-yl	0.55	n.a.	5.5
122	1	Benzimidazol-1-yl	0.78	n.a.	2.9
125	1	7-Azaindol-1-yl	0.18	n.a.	4.3

[a] IC_{50} values are the means of at least two independent determinations, errors are within ±20%.
[b] Evaluated by reversed-phase HPLC with acetonitrile/phosphate buffered saline (pH 7.4) as mobile phase
[c] 36% inhibition at 10 μM (n=2).
[d] 47% inhibition at 10 μM (n=2).
[e] 43% inhibition at 10 μM (n=2).
[f] 47% inhibition at 10 μM (n=2).

aqueous solution and against esterases present in porcine liver homogenate and porcine blood plasma. In all these environments, the compounds showed largely good stability (Table 5), as did the reference inhibitors URB597 and ABX-1431, which also possess carbamate groups. For comparison, under the conditions used, the ester prodrug oseltamivir was approximately 90% degraded in porcine liver homogenate and the ester prodrug olmesartan medoxomil was almost quantitatively hydrolyzed in porcine blood plasma.

MAGL and FAAH belong to the large group of metabolic serine hydrolases.[35] To get some information about the selectivity of the synthesized inhibitors against other serine hydrolases, we tested the compounds for inhibition of the serine hydrolases cytosolic phospholipase $A_2\alpha$ ($cPLA_2\alpha$), acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). $cPLA_2\alpha$ is known to be a key enzyme for the release of pro-inflammatory arachidonic acid from phospholipids in cells and tissues. Acetylcholinesterase plays a crucial role in nerve transmission, butyrylcholinesterase is involved in some metabolic reactions. All compounds investigated did not inhibit $cPLA_2\alpha$ and acetylcholinesterase at the highest test concentration of 10 μM (Table 5). Butyrylcholinesterase only was inhibited by the alkylcarbamates to some extent, but not by the piperidinyl carbamate derivatives.

Water solubility and permeability through the intestinal barrier are key parameters for a good bioavailability of a drug.[36] Therefore, we determined these properties for the carbamates **101** and **125**, which belong to the most active dual FAAH/MAGL and selective MAGL inhibitors, respectively, synthesized in this study. The water solubility was measured using a thermodynamic solubility assay, in which substance samples were shaken in PBS buffer (pH 7.4) at 20 °C for 20 h. After centrifugation, the supernatants were subjected to HPLC-MS analysis for quantification of the amount of compound dissolved. With this method, for compound **101** a water solubility of 6 μg/mL and for **125** of even less than 1 μg/mL was measured (Table 6). These values are well below the minimum solubility of 50 μg/mL that active substances with medium activity and average permeability should have in order to be sufficiently well absorbed.[37–38] The reference substance ABX-1431 also had only a very low solubility in water at pH 7.4. This is not surprising due to the very high lipophilicity of the substance in the uncharged state (log P of 7.4, measured by reversed-phase HPLC using acetonitrile/PBS pH 7.4 as mobile phase).

Intestinal permeability was assessed *in vitro* using a Caco-2 cell model. The substances were added to the buffer medium (pH 7.4) on the apical compartment of the cell monolayer in a concentration of 10 μM. After 2 h incubation at 37 °C, medium from the apical and the basolateral compartment of the cell monolayer was collected and the amount of compound present was analyzed by HPLC-MS. For the reference indomethacin, a permeability coefficient p_{app} of 17.5×10^{-6} cm/s was calculated. This value corresponds approximately to the value of 20.4×

Table 5. Stability of selected carbamates in different aqueous environments and inhibitory potency against some other metabolic serine hydrolases.

Comp.	PBS-buffer pH 7.4 Stability [%][a]	Porcine liver S9 fraction Stability [%][a]	Porcine blood plasma Stability [%][a]	$cPLA_2\alpha$ IC_{50} [μM][b]	AChE IC_{50} [μM][b]	BuChE IC_{50} [μM][b]
11	95±4	72±5	86±9	n.a.	n.a.	3.5[c]
13	95±3	69±5	79±8	n.a.	n.a.	1.8[c]
20	>95	75±3	82±6	n.a.	n.a.	n.a.
24	93±1	68±7	86±7	n.a.	n.a.	n.a.
101	>95	82±7	93±5	n.a.	n.a.	n.a.
125	92±7	75±8	79±6	n.a.	n.a.	n.a.
URB597 (**1**)	>95	80±4	77±6	n.a.	n.a.	>10[d]
ABX-1431 (**5**)	90±5	81±3	95±2	n.a.	n.a.	n.a.

[a] Percent of parent remaining after incubation; values are means ± standard deviations (n=3).
[b] Inhibition values of references: $cPLA_2\alpha$ inhibitor Axon-1609 IC_{50}=0.21 μM (see Supporting Information); cholinesterase inhibitor physostigmine IC_{50}= 0.18 μM (AChE) and 2.7 μM (BuChE);[34] n.a.: not active at 10 μM.
[c] Values are the means of at least two independent determinations, errors are within ±20%.
[d] 35% inhibition at 10 μM (n=2).

Table 6. Aqueous solubility, Caco-2 cell permeability, and lipophilicity (log P).

Comp.	Aqueous solubility pH 7.4 [μg/mL][a]	Permeability coefficient pH 7.4 [$\times 10^{-6}$ cm/s][b]	Log P[c]
101	6.1 ± 0.5	12.6 ± 1.0	3.4
125	< 1	10.0 ± 1.9	4.3
ABX-1431 (**5**)	< 1	n.d.[d]	7.4

[a] Values are means ± standard deviations (n = 3); for the reference indomethacin a solubility of 246 ± 18 μg/mL (n = 4) was measured under the same conditions.
[b] Values are means ± standard deviations (n = 3); for the reference indomethacin a permeability coefficient of $17.5 \pm 3.7 \times 10^{-6}$ cm/s was measured; the recoveries of the test compounds determined at the end of the experiments were as follows: > 95% for indomethacin, 70 ± 14% for **101**, 50 ± 14% for **125**, 74 ± 18% for ABX-1431 (**5**).
[c] Evaluated by reversed-phase HPLC with acetonitrile/phosphate buffered saline (pH 7.4) as mobile phase; for the reference indomethacin a log P of 2.9 was measured under the same conditions.
[d] n.d.: no permeation detectable.

10^{-6} cm/s given in the literature,[39] which was obtained under comparable conditions. The recovery of indomethacin in the two compartments was quantitative. In contrast, for the three test substances **101**, **125** and ABX-1431, recovery values of only 50–74% could be determined. This could be due to the fact that the three compounds, which all have a higher lipophilicity than indomethacin, adsorb to the plastic and the filter support and accumulate within the barrier, as has been described for highly lipophilic substances.[40] Considering these recovery values, for **101** and **125** p_{app} coefficients of 12.6×10^{-6} and 10.0×10^{-6} cm/s were measured, respectively (Table 6). Thus, these values are slightly lower than those of the reference drug indomethacin, which is classified as a well permeable drug.[39] In contrast, the reference inhibitor ABX-1431 could not be detected in the basolateral compartment after 2 h (for HPLC-chromatograms from samples of ABX-1431 and **101** taken from the apical and basolateral compartments: see Supporting Information). This finding, in combination with the very poor water solubility at pH 7.4, suggests that ABX-1431 has a very poor bioavailability. In fact, however, a relatively high bioavailability of more than 60% was determined for the substance in dogs and rats after peroral application.[22] This is possibly due to the fact that the substance that can be protonated on the piperazine ring dissolves much better at lower pH values.

Conclusion

In summary, studies on the structure-activity relationships of hexafluoroisopropyl carbamates led to a number of indolylalkyl- and azaindolylalkyl-substituted piperidine carbamates acting as inhibitors of the endocannabinoid inhibitory enzymes MAGL and FAAH, respectively. While such carbamates with a methylene linker between piperidine ring and heteroaromatic substituent like compound **20** were found to be selective or predominantly selective inhibitors of MAGL, extension of this linker by one to three carbons resulted in dual MAGL/FAAH inhibitors like compound **24**. Interestingly, the inhibitory effect of the molecules on MAGL was mainly determined by the carbamate leaving group. Different substituents on the indole ring or replacement of the indole by various azaindoles did not significantly alter the inhibitory potency. In contrast, appropriate modifications had a stronger effect on FAAH inhibition. One of the most potent dual MAGL/FAAH inhibitors was the benzimidazole derivative **101**, one of the strongest selective MAGL inhibitors was the 7-azaindole analog **125**. Both compounds showed a slightly higher MAGL inhibitory potency than the known selective MAGL inhibitor ABX-1431 and also partly surpassed this substance with regard to various physicochemical and biochemical properties such as water solubility and cell permeability.

Experimental Section

Chemistry

General

Column chromatography was performed on silica gel 60, particle size 0.040–0.063 mm, from Macherey & Nagel (Düren, Germany). Melting points were determined on a Büchi B-540 apparatus (Essen, Germany) and are uncorrected. ^{1}H NMR spectra were recorded on an DD2 spectrometer (400 MHz) or an DD2 spectrometer (600 MHz) from Agilent (Santa Clara, USA). The high resolution mass spectra (HRMS) were recorded on a Bruker (Bremen, Germany) micrOTOF–Q II spectrometer applying atmospheric pressure chemical ionization (APCI).

Preparative reversed-phase HPLC was performed using a Knauer Azura pump P2.1 L equipped with a Knauer RP18 Eurospher II 5 μm column (20 mm (I.D.) × 250 mm) protected with a RP18 Eurospher II 5 μm guard column (Knauer, Berlin, Germany) (20 mm (I.D.) × 30 mm) and eluting at a flow rate of 25 mL/min. Detection was conducted with a Shimazu SPD-6 A UV detector at 254 nm (Shimadzu Corporation, Tokyo, Japan). Chromatograms were recorded with MacDAcq32 Control Software from Bischoff (Leonberg, Germany). The compounds were dissolved in DMSO and the injected sample volume was 500 μL. The substances were obtained after distilling off the organic solvent and freeze-drying the remaining aqueous phase using a Christ alpha 1–2 LD plus apparatus (Christ, Osterode am Harz, Germany).

Purity of the target compounds was determined by reversed-phase HPLC with UV-detection on a Dionex Ultimate™ 3000 LC System (Thermo Fisher Scientific, Schwerte, Germany). The samples were prepared by mixing 20 μL of a 5 mM solution of the compound in DMSO with 180 μL of acetonitrile. 5 μL of the solutions was injected into the HPLC-system. Separation was performed using a Nucleosil 100 RP18 3 μm column (3 mm (I.D.) × 125 mm) at a flow rate of 0.4 mL/min with a gradient consisting of acetonitrile/water/trifluoroacetic acid (42:58:0.1 to 86:14:0.1, v/v/v). UV-absorbance was measured at 254 nm. Under these conditions all compounds showed purities greater or equal to 95%.

For the synthesis of the target compounds not described below: See Supporting Information.

1,1,1,3,3,3-Hexafluoropropan-2-yl *N*-[5-(6-fluoroindol-1-yl)pentyl]carbamate (11)

A solution of 1,1,1,3,3,3-hexafluoropropan-2-ol (127 μL, 203 mg, 1.21 mmol) in dry acetonitrile (5 mL) was treated with triethylamine (850 μL, 617 mg, 6.10 mmol), followed by bis(pentafluorophenyl) carbonate (494 mg, 1.25 mmol). The mixture was stirred at 0 °C for 30 min, and after warming to room temperature for an additional 2 h. Subsequently, a solution of 5-(6-fluoroindol-1-yl)pentan-1-amine (**10**)[23] (180 mg, 0.817 mmol) and triethylamine (566 μL, 411 mg, 4.06 mmol) in dry acetonitrile (5 mL) was added. The resulting mixture was stirred at room temperature overnight. After evaporation under reduced pressure, the residue was first cleaned up by chromatography on silica gel (cyclohexane/ethyl acetate, 8:2) and then further purified by preparative RP-HPLC (acetonitrile/H_2O/formic acid, 8:2:0.1) to yield **11** (131 mg, 39%) as a solid. $C_{17}H_{17}F_7N_2O_2$ (414.3); mp 76–77 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.27–1.37 (m, 2H), 1.51–1.58 (m, 2H), 1.80–1.89 (m, 2H), 3.17–3.24 (m, 2H), 4.05 (t, J = 7.0 Hz, 2H), 5.65 (hept, J = 6.2 Hz, 1H), 6.45 (dd, J = 3.2 Hz and 0.9 Hz, 1H), 6.85 (ddd, J = 9.6 Hz, 8.6 Hz and 2.3 Hz, 1H), 6.97 (dd, J = 9.9 Hz and 2.3 Hz, 1H), 7.03 (d, J = 3.2 Hz, 1H), 7.51 (dd, J = 8.6 Hz and 5.5 Hz, 1H); ^{13}C (101 MHz, $CDCl_3$): δ (ppm) 24.03, 29.37, 29.70, 41.54, 46.44, 67.23–68.32 (m, F_3C-CH-CF_3), 95.79 (d, $J_{C\text{-}F}$ = 26.0 Hz), 101.52, 108.24 (d, $J_{C\text{-}F}$ = 24.5 Hz), 121.81, 125.15, 128.22, 136.01, 152.65, 159.90 (d, $J_{C\text{-}F}$ = 236.9 Hz); HRMS (APCI, direct probe) *m/z* $[M+H]^+$ calculated: 415.1251, found: 415.1313.

1,1,1,3,3,3-Hexafluoropropan-2-yl *N*-[6-(6-fluoroindol-1-yl)hexyl]carbamate (13)

6-(6-Fluoroindol-1-yl)hexan-1-amine (**12**)[24] (250 mg, 1.07 mmol) was treated with bis(pentafluorophenyl) carbonate (641 mg, 1.63 mmol) and 1,1,1,3,3,3-hexafluoropropan-2-ol (166 μL, 265 mg, 1.58 mmol) in the same manner as described for the preparation of **11**. The reaction product was first purified by chromatography on silica gel (cyclohexane/ethyl acetate, 8:2) to obtain 330 mg of **13**. An aliquot (100 mg) of this crude product was further cleaned up by preparative RP-HPLC (acetonitrile/H_2O/formic acid, 8:2:0.1) to yield **13** (67 mg) as a solid. $C_{18}H_{19}F_7N_2O_2$ (428.4); mp 59–60 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.29–1.38 (m, 4H), 1.47–1.55 (m, 2H), 1.78–1.88 (m, 2H), 3.18–3.25 (m, 2H), 4.06 (t, J = 7.0 Hz, 2H), 5.67 (hept, J = 6.2 Hz, 1H), 6.46 (dd, J = 3.1 Hz and 0.9 Hz, 1H), 6.86 (ddd, J = 9.5 Hz, 8.6 Hz and 2.3 Hz, 1H), 6.99 (dd, J = 10.0 Hz and 2.3 Hz, 1H), 7.05 (d, J = 3.1 Hz, 1H), 7.52 (dd, J = 8.6 Hz and 5.4 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) 26.32, 26.67, 29.56, 30.02, 41.66, 46.56, 67.30–68.19 (m, F_3C-CH-CF_3), 95.82 (d, $J_{C\text{-}F}$ = 26.2 Hz), 101.39, 108.18 (d, $J_{C\text{-}F}$ = 24.7 Hz), 121.75, 125.12, 128.25, 136.03, 152.62, 159.87 (d, $J_{C\text{-}F}$ = 237.3 Hz); HRMS (APCI, direct probe) *m/z* $[M+H]^+$ calculated: 429.1408, found: 429.1457.

Benzyl 4-[(6-fluoroindol-1-yl)methyl]piperidine-1-carboxylate (18)

A solution of 6-fluoroindole (344 mg, 2.55 mmol) in dry DMF (10 mL) was treated with sodium hydride (60% dispersion in mineral oil) (127 mg, 3.18 mmol) and stirred at room temperature for 30 min. The resulting suspension was added dropwise to a solution of benzyl 4-{[(methylsulfonyl)oxy]methyl}piperidine-1-carboxylate (**17**) (793 mg, 2.42 mmol) in dry DMF (10 mL). After stirring at room temperature overnight, the mixture was diluted with brine (25 mL) and extracted exhaustively with ethyl acetate. The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified by chromatography on silica gel (cyclohexane/ethyl acetate, 7:3) to yield **18** (536 mg, 60%) as an oil. $C_{22}H_{23}FN_2O_2$ (366.4); ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.15–1.30 (m, 2H), 1.55–1.57 (m, 2H), 1.95–2.07 (m, 1H), 2.70 (t, J = 13.0 Hz, 2H), 3.93 (d, J = 7.2 Hz, 2H), 4.12–4.29 (m, 2H), 5.11 (s, 2H), 6.46 (dd, J = 3.2 Hz and 0.9 Hz, 1H), 6.86 (ddd, J = 9.6 Hz, 8.6 Hz and 2.3 Hz, 1H), 6.97 (dd, J = 10.0 Hz and 2.3 Hz, 1H), 7.01 (d, J = 3.2 Hz, 1H), 7.27–7.38 (m, 5H), 7.52 (dd, J = 8.6 Hz and 5.4 Hz, 1H); HRMS (APCI, direct probe) *m/z* $[M+H]^+$ calculated: 367.1816, found: 367.1821.

6-Fluoro-1-(piperidin-4-ylmethyl)indole (19)

Compound **18** (530 mg, 1.45 mmol) was dissolved in methanol/THF (1:1) (5 mL) and treated with a catalytic amount of palladium (10%) on activated charcoal (53 mg). The mixture was stirred under a balloon filled with hydrogen at room temperature for 4 h. After filtration through Celite 545, the solvent was evaporated under reduced pressure to obtain **19** (269 mg, 80%) as an oil. $C_{14}H_{17}FN_2$ (232.3); ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.19–1.30 (m, 2H), 1.53–1.61 (m, 2H), 1.88–2.01 (m, 1H), 2.49–2.59 (m, 2H), 3.04–3.12 (m, 2H), 3.91 (d, J = 7.2 Hz, 2H), 6.45 (dd, J = 3.2 Hz and 0.9 Hz, 1H), 6.86 (ddd, J = 9.7 Hz, 8.6 Hz and 2.3 Hz, 1H), 6.99 (dd, J = 10.0 Hz and 2.2 Hz, 1H), 7.03 (d, J = 3.2 Hz, 1H), 7.51 (dd, J = 8.7 Hz and 5.4 Hz, 1H); HRMS (APCI, direct probe) *m/z* $[M+H]^+$ calculated: 233.1449, found: 233,1455.

1,1,1,3,3,3-Hexafluoropropan-2-yl 4-[(6-fluoroindol-1-yl)methyl]piperidine-1-carboxylate (20)

Compound **19** (102 mg, 0.44 mmol) was treated with bis(pentafluorophenyl) carbonate (261 mg, 0.66 mmol) and 1,1,1,3,3,3-hexafluoropropan-2-ol (69 μL, 110 mg, 0.65 mmol) in the same manner as described for the preparation of **11**. The reaction product was purified by chromatography on silica gel (cyclohexane/ethyl acetate, 8:2) to yield **20** (30 mg, 16%) as an oil. $C_{18}H_{17}F_7N_2O_2$ (426.3); ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.21–1.35 (m, 2H), 1.62–1.67 (m, 2H), 2.03–2.11 (m, 1H), 2.76–2.88 (m, 2H), 3.96 (d, J = 7.3 Hz, 2H), 4.13–4.23 (m, 2H), 5.74 (hept, J = 6.2 Hz, 1H), 6.48 (dd, J = 3.1 Hz and 0.9 Hz, 1H), 6.88 (ddd, J = 9.5 Hz, 8.6 Hz and 2.3 Hz, 1H), 6.98 (dd, J = 9.9 Hz and 2.3 Hz, 1H), 7.02 (d, J = 3.2 Hz, 1H), 7.53 (dd, J = 8.6 Hz and 5.3 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) 29.69, 30.05, 37.04, 44.28, 44.90, 52.08, 67.73–68.73 (m, F_3C-CH-CF_3), 95.87 (d, $J_{C\text{-}F}$ = 26.1 Hz), 101.76, 108.45 (d, $J_{C\text{-}F}$ = 24.4 Hz), 121.96 (d, $J_{C\text{-}F}$ = 10.2 Hz), 125.13, 128.78 (d, $J_{C\text{-}F}$ = 3.6 Hz), 136.18 (d, $J_{C\text{-}F}$ = 11.9 Hz), 151.50, 159.97 (d, $J_{C\text{-}F}$ = 237.8 Hz); HRMS (APCI, direct probe) *m/z* $[M+H]^+$ calculated: 427.1251, found: 427.1354.

1,1,1,3,3,3-Hexafluoropropan-2-yl 4-[2-(6-fluoroindol-1-yl)ethyl]piperidine-1-carboxylate (24)

6-Fluoro-1-[2-(piperidin-4-yl)ethyl]indole (**23**)[24] (170 mg, 0.69 mmol) was treated with bis(pentafluorophenyl) carbonate (416 mg, 1.06 mmol) and 1,1,1,3,3,3-hexafluoropropan-2-ol (107 μL, 171 mg, 1.02 mmol) in the same manner as described for the preparation of **11**. The reaction product was first purified by chromatography on silica gel (cyclohexane/ethyl acetate, 8:2). Further purification by preparative RP-HPLC (acetonitrile/H_2O/formic acid, 8:2:0.1) yielded **24** (140 mg, 46%) as an oil. $C_{19}H_{19}F_7N_2O_2$ (440.4); ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.17–1.31 (m, 2H), 1.41–1.56 (m, 1H), 1.73–1.85 (m, 4H), 2.77–2.92 (m, 2H), 4.08–4.21 (m, 4H), 5.75 (hept, J = 6.4 Hz, 1H), 6.46 (dd, J = 3.2 Hz and 0.9 Hz, 1H), 6.86 (ddd, J = 9.5 Hz, 8.6 Hz and 2.2 Hz, 1H), 6.96 (dd, J = 10.0 Hz and 2.2 Hz, 1H), 7.03 (d, J = 3.2 Hz, 1H), 7.52 (dd, J = 8.7 Hz and 5.4 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) 31.06, 31.98, 33.32, 36.38, 43.95, 44.58, 45.19, 67.67–68.74 (m, F_3C-CH-CF_3), 95.68 (d, $J_{C\text{-}F}$ = 26.1 Hz), 101.77, 108.35 (d, $J_{C\text{-}F}$ = 24.7 Hz), 121.93 (d, $J_{C\text{-}F}$ = 9.9 Hz), 125.15, 127.95 (d, $J_{C\text{-}F}$ = 3.5 Hz), 135.94 (d, $J_{C\text{-}F}$ =

11.3 Hz), 151.54, 159.93 (d, $J_{C\text{-}F}$ = 237.6 Hz); HRMS (APCI, direct probe) *m/z* $[M+H]^+$ calculated: 441.1408, found: 441.1389.

Methyl *N*-[5-(6-fluoroindol-1-yl)pentyl]carbamate (33)

Triethylamine (1.36 mL, 987 mg, 9.76 mmol) and methyl chloroformate (164 µL, 201 mg, 2.12 mmol) were slowly added to an ice-cooled solution of 5-(6-fluoroindol-1-yl)pentan-1-amine (**10**)[23] (360 mg, 1.63 mmol) in dry dichloromethane (5 mL). The mixture was stirred at 0 °C for 30 min, allowed to warm up to room temperature and stirred at this temperature for another 2 h. The solvent was evaporated under reduced pressure and the residue purified by chromatography on silica gel (cyclohexane/ethyl acetate, 8:2) to obtain **33** (358 mg, 79%) as an oil. $C_{15}H_{19}FN_2O_2$ (278.3); ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.29–1.36 (m, 2H), 1.51 (pent, *J* = 7.2 Hz, 2H), 1.84 (pent, *J* = 7.4 Hz, 2H), 3.08–3.18 (m, 2H), 3.65 (s, 3H), 4.05 (t, *J* = 7.0 Hz, 2H), 6.45 (dd, *J* = 3.2 Hz and 0.9 Hz, 1H), 6.85 (ddd, *J* = 9.5 Hz, 8.6 Hz and 2.3 Hz, 1H), 6.98 (dd, *J* = 10.1 Hz and 2.3 Hz, 1H), 7.05 (d, *J* = 3.2 Hz, 1H), 7.51 (dd, *J* = 8.6 Hz and 5.4 Hz, 1H); HRMS (APCI, direct probe) *m/z* $[M+H]^+$ calculated: 279.1503, found: 279.1492.

5-(6-Fluoroindol-1-yl)-*N*-methylpentan-1-amine (34)

An ice-cooled solution of **33** (350 mg, 1.26 mmol) in dry THF (5 mL) was treated slowly with $LiAlH_4$ solution (1 M in THF) (6.29 mL, 6.29 mmol). After warming up to room temperature, the mixture was stirred for 3 h at this temperature and subsequently heated under reflux for 2 h. The reaction mixture was cooled to 0 °C and quenched with H_2O (3 mL), followed by aqueous NaOH solution (0.1 M) (3 mL). The resulting suspension was filtered over Celite 545 and the filter cake was washed with ethyl acetate. The filtrate was treated with saturated $NaHCO_3$ solution (25 mL) and exhaustively extracted with ethyl acetate. The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to yield **34** (231 mg, 78%) as an oil. $C_{14}H_{19}FN_2$ (234.3); ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.32–1.41 (m, 2H), 1.51–1.59 (m, 2H), 1.80–1.89 (m, 2H), 2.43 (s, 3H), 2.58 (t, *J* = 7.0 Hz, 2H), 4.05 (t, *J* = 7.1 Hz, 2H), 6.45 (dd, *J* = 3.2 Hz and 0.9 Hz, 1H), 6.86 (ddd, *J* = 9.7 Hz, 8.6 Hz and 2.3 Hz, 1H), 7.00 (dd, *J* = 10.0 Hz and 2.3 Hz, 1H), 7.06 (d, *J* = 3.2 Hz, 1H), 7.51 (dd, *J* = 8.6 Hz and 5.4 Hz, 1H); HRMS (APCI, direct probe) m/z $[M+H]^+$ calculated: 235.1605, found: 235.1615.

1,1,1,3,3,3-Hexafluoropropan-2-yl *N*-methyl-*N*-[5-(6-fluoroindol-1-yl)pentyl]carbamate (35)

Compound **34** (100 mg, 0.43 mmol) was treated with bis(pentafluorophenyl) carbonate (252 mg, 0.64 mmol) and 1,1,1,3,3,3-hexafluoropropan-2-ol (66 µL, 105 mg, 0.63 mmol) in the same manner as described for the preparation of **11**. The reaction product was first purified by chromatography on silica gel (cyclohexane/ethyl acetate, 8:2). Further purification by preparative RP-HPLC (acetonitrile/H_2O/formic acid, 8:2:0.1) yielded **35** (23 mg, 13%) as an oil. $C_{18}H_{19}F_7N_2O_2$ (428.4); ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) 1.28–1.33 (m, 2H), 1.53–1.61 (m, 2H), 1.82–1.90 (m, 2H), 2.91–2.94 (m, 3H), 3.26–3.32 (m, 2H), 4.06 (t, *J* = 7.0 Hz, 2H), 5.75 (hept, *J* = 6.9 Hz, 1H), 6.45 (dd, *J* = 3.2 Hz and 0.9 Hz, 1H), 6.85 (ddd, *J* = 9.6 Hz, 8.6 Hz and 2.3 Hz, 1H), 6.97 (dd, *J* = 9.9 Hz and 2.3 Hz, 1H), 7.03 (d, *J* = 3.2 Hz, 1H), 7.51 (dd, *J* = 8.6 Hz and 5.5 Hz, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ (ppm) 23.93, 29.76, 29.82, 34.15, 46.51, 49.04, 67.06–68.80, 95.68 (d, $J_{C\text{-}F}$ = 26.0 Hz), 101.44, 108.10 (d, $J_{C\text{-}F}$ = 24.5 Hz), 121.76, 125.13, 128.26, 135.99, 152.51, 159.87 (d, $J_{C\text{-}F}$ = 237.2 Hz); HRMS (APCI, direct probe) *m/z* $[M+H]^+$ calculated: 429.1408, found: 429.1415.

1-(5-Bromopentyl)-6-fluoroindole (36)

Sodium hydride (60% dispersion in mineral oil) (360 mg, 9.00 mmol) was carefully added to a solution of 6-fluoroindole (811 mg, 6.00 mmol) in dry DMF (20 mL). The suspension was stirred at room temperature for 30 min until no further formation of hydrogen could be observed. Then a solution of 1,5-dibromopentane (2.97 mL, 5.01 g, 21.8 mmol) in dry DMF (20 mL) was added and the resulting mixture was stirred at 90 °C for 3 h. The solvent was evaporated under reduced pressure, the residue was suspended in H_2O and the aqueous mixture was exhaustively extracted with ethyl acetate. The combined organic layers were dried over anhydrous Na_2SO_4, filtered and evaporated to dryness. Purification of the residue was carried out by chromatography on silica gel (cyclohexane to cyclohexane/ethyl acetate, 19:1) to yield **36** (1.28 g, 75%) as an oil. $C_{13}H_{15}BrFN$ (284.2); ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.43–1.51 (m, 2H), 1.81–1.91 (m, 4H), 3.38 (t, *J* = 6.7 Hz, 2H), 4.08 (t, *J* = 7.0 Hz, 2H), 6.47 (dd, *J* = 3.2 Hz and 0.9 Hz, 1H), 6.87 (ddd, *J* = 9.7 Hz, 8.6 Hz and 2.3 Hz, 1H), 7.00 (dd, *J* = 9.9 Hz and 2.2 Hz, 1H), 7.07 (d, *J* = 3.2 Hz, 1H), 7.53 (dd, *J* = 8.6 Hz and 5.4 Hz, 1H); HRMS (APCI, direct probe) *m/z* $[M+H]^+$ calculated: 284.0445, found: 284.0439.

N-Ethyl-5-(6-fluoroindol-1-yl)pentan-1-amine (37)

A solution of **36** (250 mg, 0.88 mmol) in dry DMF (10 mL) was treated with ethylamine (299 µL, 203 mg, 4.50 mmol) and cesium carbonate (464 mg, 1.42 mmol) and stirred at room temperature for 24 h. After dilution with water and adjusting the pH value to 10 with aqueous NaOH solution (1 M), the mixture was exhaustively extracted with ethyl acetate. The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. Purification by chromatography on silica gel (ethyl acetate/triethylamine, 100:0.1) yielded **37** (176 mg, 81%) as an oil. $C_{15}H_{21}FN_2$ (248.3); ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) 1.15 (t, *J* = 7.1 Hz, 3H), 1.32–1.38 (m, 2H), 1.59 (pent, *J* = 7.5 Hz, 2H), 1.80–1.87 (m, 2H), 2.63 (t, *J* = 7.5 Hz, 2H), 2.69 (q, *J* = 7.2 Hz, 2H), 4.05 (t, *J* = 7.1 Hz, 2H), 6.45 (dd, *J* = 3.1 Hz and 0.9 Hz, 1H), 6.86 (ddd, *J* = 9.6 Hz, 8.4 Hz and 2.3 Hz, 1H), 6.99 (dd, *J* = 9.9 Hz and 2.3 Hz, 1H), 7.06 (d, *J* = 3.2 Hz, 1H), 7.51 (dd, *J* = 8.6 Hz and 5.4 Hz, 1H); HRMS (APCI, direct probe) *m/z* $[M+H]^+$ calculated: 249.1762, found: 249.1773.

1,1,1,3,3,3-Hexafluoropropan-2-yl *N*-ethyl-*N*-[5-(6-fluoroindol-1-yl)pentyl]carbamate (38)

Compound **37** (80 mg, 0.32 mmol) was treated with bis(pentafluorophenyl) carbonate (190 mg, 0.48 mmol) and 1,1,1,3,3,3-hexafluoropropan-2-ol (50 µL, 80 mg, 0.48 mmol) in the same manner as described for the preparation of **11**. The reaction product was first purified by chromatography on silica gel (cyclohexane/ethyl acetate, 9:1). Further purification by preparative RP-HPLC (acetonitrile/H_2O/formic acid, 8:2:0.1) yielded **38** (30 mg, 21%) as an oil. $C_{19}H_{21}F_7N_2O_2$ (442.4); ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.10–1.17 (m, 3H), 1.25–1.36 (m, 2H), 1.51–1.63 (m, 2H), 1.79–1.91 (m, 2H), 3.20–3.34 (m, 4H), 4.06 (t, *J* = 7.0 Hz, 2H), 5.76 (hept, *J* = 6.4 Hz, 1H), 6.46 (dd, *J* = 3.1 Hz and 1.1 Hz, 1H), 6.88 (ddd, *J* = 9.5 Hz, 8.5 Hz and 2.3 Hz, 1H), 7.01 (ddd, *J* = 10.0 Hz, 6.9 Hz and 2.3 Hz, 1H), 7.05 (d, *J* = 3.1 Hz, 1H), 7.52 (dd, *J* = 8.5 Hz and 5.4 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) 13.77, 24.14, 27.63, 29.79, 42.46, 43.59, 46.52, 67.11–68.91 (m, F_3C-CH-CF_3), 95.69 (d, $J_{C\text{-}F}$ = 26.0 Hz), 101.46, 108.07 (d, $J_{C\text{-}F}$ = 24.5 Hz), 121.75, 125.15, 128.26, 136.00, 151.86, 159.67 (d, $J_{C\text{-}F}$ = 237.4 Hz); HRMS (APCI, direct probe) *m/z* $[M+H]^+$ calculated: 443.1564, found: 443.1570.

6-Fluoro-1-tosylindole (88)

A solution of 6-fluoroindole (200 mg, 1.48 mmol) in dry DMF (5 mL) was treated with sodium hydride (60% dispersion in mineral oil) (71 mg, 1.78 mmol). After stirring at room temperature until no further formation of hydrogen was observed, toluene-4-sulfonyl chloride (282 mg, 1.48 mmol) was added and the mixture was stirred at room temperature for 16 h. The reaction mixture was diluted with water and exhaustively extracted with ethyl acetate. The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by chromatography on silca gel (cyclohexane/ethyl acetate, 7:3) to yield **88**[29] (331 mg, 77%) as an oil. $C_{15}H_{12}FNO_2S$ (289.3); ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 2.36 (s, 3H), 6.62 (dd, J = 3.7 Hz and 0.9 Hz, 1H), 6.98 (ddd, J = 9.2 Hz, 8.6 Hz and 2.3 Hz, 1H), 7.22–7.26 (m, 2H), 7.44 (dd, J = 8.7 Hz and 5.3 Hz, 1H), 7.53 (d, J = 3.7 Hz, 1H), 7.71 (dd, J = 9.7 Hz and 2.4 Hz, 1H), 7.74–7.79 (m, 2H); HRMS (APCI, direct probe) *m/z* $[M+H]^+$ calculated: 290.0646, found: 290.0634.

Benzyl 4-[2-(6-fluoroindol-1-yl)ethyl]piperazine-1-carboxylate (90)

A solution of benzyl 4-(2-hydroxyethyl)piperazine-1-carboxylate (**89**)[41] (251 mg, 0.95 mmol) in dry DMF (10 mL) was treated with potassium *tert*-butylate (128 mg, 1.14 mmol) and stirred at room temperature for 15 min. After addition of 6-fluoro-1-tosylindole (**88**) (330 mg, 1.14 mmol), the solution was heated under vigorous stirring at 90 °C for 24 h. Subsequently, the mixture was cooled to room temperature, diluted with water and exhaustively extracted with ethyl acetate. The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated. Purification of the residue by chromatography on silica gel (cyclohexane/ethyl acetate, 8:2) yielded **90** (61 mg, 17%) as an oil. $C_{22}H_{24}FN_3O_2$ (381.5); ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 2.33–2.54 (m, 4H), 2.67–2.83 (m, 2H), 3.42–3.57 (m, 4H), 4.14 (t, J = 6.9 Hz, 2H), 5.12 (s, 2H), 6.46 (dd, J = 3.1 Hz and 0.9 Hz, 1H), 6.87 (ddd, J = 9.9 Hz, 8.9 Hz and 2.3 Hz, 1H), 7.02 (dd, J = 9.8 Hz and 2.2 Hz, 1H), 7.11 (d, J = 3.3 Hz, 1H), 7.27–7.38 (m, 5H), 7.51 (dd, J = 8.7 Hz and 5.4 Hz, 1H); HRMS (APCI, direct probe) *m/z* $[M+H]^+$ calculated: 382.1925, found: 382.1925.

6-Fluoro-1-[2-(piperazin-1-yl)ethyl]indole (91)

Compound **90** (60 mg, 0.16 mmol) was hydrogenated in the same manner as described above for the synthesis of **19** to yield **91** (39 mg, quantitative) as an oil. $C_{14}H_{18}FN_3$ (247.3); ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 2.41–2.66 (m, 4H), 2.77 (t, J = 6.6 Hz, 2H), 3.01–3.26 (m, 4H), 4.16 (t, J = 6.6 Hz, 2H), 6.45 (dd, J = 3.2 Hz and 0.8 Hz, 1H), 6.86 (ddd, J = 9.8 Hz, 8.6 Hz and 2.3 Hz, 1H), 6.99 (dd, J = 10.1 Hz and 2.2 Hz, 1H), 7.07 (d, J = 3.2 Hz, 1H), 7.51 (dd, J = 8.7 Hz and 5.4 Hz, 1H); HRMS (APCI, direct probe) *m/z* $[M+H]^+$ calculated: 248.1558, found: 248.1558.

1,1,1,3,3,3-Hexafluoropropan-2-yl 4-[2-(6-fluoroindol-1-yl)ethyl]piperazine-1-carboxylate (92)

Compound **91** (39 mg, 0.16 mmol) was treated with bis(pentafluorophenyl) carbonate (96 mg, 0.24 mmol) and 1,1,1,3,3,3-hexafluoropropan-2-ol (25 μL, 41 mg, 0.24 mmol) in the same manner as described for the preparation of **11**. The reaction product was first purified by chromatography on silica gel (cyclohexane/ethyl acetate, 8:2). Further purification by preparative RP-HPLC (acetonitrile/H_2O/formic acid, 7:3:0.1) yielded **92** (29 mg, 44%) as a solid. $C_{18}H_{18}F_7N_3O_2$ (441.4); mp 67–69 °C; ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) 2.39–2.54 (m, 4H), 2.71–2.83 (m, 2H), 3.47–3.61 (m, 4H), 4.18 (t, J = 6.7 Hz, 2H), 5.74 (hept, J = 6.4 Hz, 1H, F_3C–C<u>H</u>–CF_3), 6.47 (dd, J = 3.3 Hz and 0.9 Hz, 1H), 6.88 (ddd, J = 9.5 Hz, 8.6 Hz and 2.4 Hz, 1H), 7.02 (dd, J = 10.1 Hz and 2.3 Hz, 1H), 7.11 (d, J = 3.2 Hz, 1H), 7.53 (dd, J = 8.5 Hz and 9.2 Hz, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ (ppm) 44.47, 44.67, 44.77, 52.88, 53.04, 57.52, 67.20–68.98 (m, F_3C–<u>C</u>H–CF_3), 95.76 (d, $J_{C\text{-}F}$ = 26.5 Hz), 101.80, 108.34 (d, $J_{C\text{-}F}$ = 19.0 Hz), 121.82 (d, $J_{C\text{-}F}$ = 9.9 Hz), 125.20, 128.50 (d, $J_{C\text{-}F}$ = 3.9 Hz), 135.96 (d, $J_{C\text{-}F}$ = 11.2), 151.44, 159.94 (d, $J_{C\text{-}F}$ = 236.3 Hz); HRMS (APCI, direct probe) *m/z* $[M+H]^+$ calculated: 442.1360, found: 442.1365.

Benzyl 4-[2-(benzimidazol-1-yl)ethyl]piperidine-1-carboxylate (99)

A solution of benzimidazole (69 mg, 0.58 mmol) in dry DMF (5 mL) was treated with sodium hydride (60% dispersion in mineral oil) (29 mg, 0.73 mmol). After stirring at room temperature until no further formation of hydrogen was observed, the suspension was added dropwise to a solution of **21** (200 mg, 0.59 mmol) in dry DMF (5 mL). The mixture was stirred at room temperature overnight, diluted with brine and exhaustively extracted with ethyl acetate. The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified by chromatography on silica gel (ethyl acetate/triethylamine 100:0.1) to yield **99** (206 mg, 97%) as an oil. $C_{22}H_{25}N_3O_2$ (363.5); ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.17–1.27 (m, 2H), 1.41–1.53 (m, 1H), 1.65–1.78 (m, 2H), 1.81–1.90 (m, 2H), 2.67–2.81 (m, 2H), 4.13–4.26 (m, 4H), 5.12 (s, 2H), 7.29–7.33 (m, 2H), 7.33–7.36 (m, 5H), 7.36–7.40 (m, 1H), 7.80–7.84 (m, 1H), 7.96 (s, 1H); HRMS (APCI, direct probe) *m/z* $[M+H]^+$ calculated: 364.2020, found: 364.2044.

1-[2-(Piperidin-4-yl)ethyl]benzimidazole (100)

Compound **99** (205 mg, 0.56 mmol) was hydrogenated in the same manner as described above for the synthesis of **19** to yield **100** (128 mg, 99%) as an oil. $C_{14}H_{19}N_3$ (229.3); ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.19–1.32 (m, 2H), 1.36–1.47 (m, 1H), 1.68–1.76 (m, 2H), 1.80–1.88 (m, 2H), 2.53–2.63 (m, 2H), 3.05–3.13 (m, 2H), 4.21 (t, J = 7.4 Hz, 2H), 7.27–7.31 (m, 2H), 7.36–7.41 (m, 1H), 7.79–7.82 (m, 1H), 7.89 (s, 1H); HRMS (APCI, direct probe) *m/z* $[M+H]^+$ calculated: 230.1652, found: 230.1646.

1,1,1,3,3,3-Hexafluoropropan-2-yl 4-[2-(benzimidazol-1-yl)ethyl]piperidine-1-carboxylate (101)

Compound **100** (120 mg, 0.52 mmol) was treated with bis(pentafluorophenyl) carbonate (309 mg, 0.78 mmol) and 1,1,1,3,3,3-hexafluoropropan-2-ol (82 μL, 131 mg, 0.78 mmol) in the same manner as described for the preparation of **11**. The reaction product was first purified by chromatography on silica gel (cyclohexane/ethyl acetate, 8:2). Further purification by preparative RP-HPLC (acetonitrile/H_2O/triethylamine, 7:3:0.1) yielded **101** (73 mg, 33%) as an oil. $C_{18}H_{19}F_6N_3O_2$ (423.4); ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.20–1.37 (m, 2H), 1.50–1.60 (m, 1H), 1.78–1.86 (m, 2H), 1.88–1.97 (m, 2H), 2.78–2.94 (m, 2H), 4.12–4.24 (m, 2H), 4.33 (t, J = 7.5 Hz, 2H), 5.74 (hept, J = 6.3 Hz, 1H), 7.35–7.39 (m, 2H), 7.41–7.47 (m, 1H), 7.88 (d, J = 7.1 Hz, 1H), 8.46 (s, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ (ppm) 31.52, 31.88, 33.19, 36.15, 43.16, 44.48, 45.08, 67.19–69.25 (m, F_3C-<u>C</u>H-CF_3), 110.16, 119.58, 123.71, 124.19, 132.98, 140.57, 142.31, 151.49; HRMS (APCI, direct probe) *m/z* $[M+H]^+$ calculated: 424.1454, found: 424.1466.

Benzyl 4-[(7-azaindol-1-yl)methyl]piperidine-1-carboxylate (123)

7-Azaindole (65 mg, 0.55 mmol) was reacted with **17** (200 mg, 0.61 mmol) in the same manner as described for the synthesis of **18**. The crude product was purified by chromatography on silica gel (ethyl acetate/triethylamine 100:0.1) to yield **123** (124 mg, 65%) as an oil. $C_{21}H_{23}N_3O_2$ (349.4); ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.20–1.30 (m, 2H), 1.51–1.65 (m, 2H), 2.10–2.23 (m, 1H), 2.67–2.81 (m, 2H), 4.11–4.27 (m, 4H), 5.11 (s, 2H), 6.48 (d, J=3.4 Hz, 1H), 7.11 (dd, J=7.4 Hz and 5.1 Hz, 1H), 7.17 (d, J=3.5 Hz, 1H), 7.28–7.38 (m, 5H), 7.97 (dd, J=7.8 Hz and 1.5 Hz, 1H), 8.33 (dd, J=4.8 Hz and 1.6 Hz, 1H); HRMS (APCI, direct probe) *m/z* $[M+H]^+$ calculated: 350.1863, found: 350.1924.

1-(Piperidin-4-ylmethyl)-7-azaindole (124)

Compound **123** (120 mg, 0.34 mmol) was hydrogenated in the same manner as described for the synthesis of **19** to yield **124** (68 mg, 92%) as an oil. $C_{13}H_{17}N_3$ (215.3); ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.25–1.35 (m, 2H), 1.58–1.63 (m, 2H), 2.07–2.15 (m, 1H), 2.54–2.61 (m, 2H), 3.08–3.14 (m, 2H), 4.16 (d, J=7.4 Hz, 2H), 6.44 (d, J=3.5 Hz, 1H), 7.05 (dd, J=7.8 Hz and 4.7 Hz, 1H), 7.17 (d, J=3.5 Hz, 1H), 7.90 (dd, J=7.8 Hz and 1.6 Hz, 1H), 8.31 (dd, J=4.7 Hz and 1.5 Hz, 1H); HRMS (APCI, direct probe) *m/z* $[M+H]^+$ calculated: 216.1495, found: 216.1524.

1,1,1,3,3,3-Hexafluoropropan-2-yl 4-[(7-azaindol-1-yl)methyl]piperidine-1-carboxylate (125)

Compound **124** (65 mg, 0.30 mmol) was treated with bis(pentafluorophenyl) carbonate (192 mg, 0.49 mmol) and 1,1,1,3,3,3-hexafluoropropan-2-ol (51 μL, 81 mg, 0.48 mmol) in the same manner as described for the preparation of **11**. The reaction product was first purified by chromatography on silica gel (cyclohexane/ethyl acetate, 8:2) and further cleaned up by preparative RP-HPLC (acetonitrile/H_2O/triethylamine, 8:2:0.1) to yield **125** (46 mg, 37%) as a solid. $C_{17}H_{17}F_6N_3O_2$ (409.3); mp 122–123 °C; ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) 1.26–1.36 (m, 2H), 1.64–1.70 (m, 2H), 2.16–2.29 (m, 1H), 2.76–2.88 (m, 2H), 4.14–4.19 (m, 2H), 4.25 (d, J=7.3 Hz, 2H), 5.74 (hept, J=6.3 Hz, 1H), 6.50 (d, J=3.4 Hz, 1H), 7.13 (dd, J=7.5 Hz and 3.4 Hz, 1H), 7.17 (d, J=3.5 Hz, 1H), 7.99 (dd, J=7.9 Hz and 1.5 Hz, 1H), 8.31 (dd, J=4.9 Hz and 1.5 Hz, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ (ppm) 29.42, 29.77, 36.81, 44.27, 44.89, 50.56, 67.20–69.02 (m, F_3C-CH-CF_3), 100.16, 115.93, 121.77, 129.25, 130.21, 141.98, 146.51, 151.51; HRMS (APCI, direct probe) *m/z* $[M+H]^+$ calculated: 410.1298, found: 410.1373.

Biochemical and Physicochemical Analysis

Inhibition of Fatty Acid Amide Hydrolase (FAAH)

Inhibition of FAAH was measured according to a published method.[24] Briefly, a DMSO solution of the inhibitor or in case of the controls DMSO alone was added to a DMSO solution of the substrate *N*-(2-hydroxyethyl)-4-pyren-1-ylbutanamide. The mixture was treated with a homogenate of rat brain prepared with potassium phosphate buffer containing EDTA and then diluted with a solution of Triton X-100 (0.2%) in phosphate buffered saline (PBS). In the final incubation volume of 100 μL, the pyrenylbutanamide substrate concentration was 100 μM. After incubation at 37 °C for 60 min, the enzyme reaction was terminated by addition of 200 μL acetonitrile/methanol (1:1, v/v) containing the internal standard 6-pyren-1-ylhexanoic acid. After centrifugation, the amount of 4-pyren-1-ylbutanoic acid released by the enzyme was determined in the supernatant by reversed-phase HPLC with fluorescence detection. In parallel, blank incubations were performed in the absence of the enzyme and evaluated analogously. For the determination of the IC_{50} values, the ratios of the peak areas of enzyme product and internal standard obtained at different inhibitor concentrations, corrected by the corresponding value of the blank incubations, were used. The calculation of the IC_{50} values was carried out with the probit transformation.[42] The reference inhibitor URB597 was purchased from Cayman Chemical (via Biomol, Hamburg, Germany).

Inhibition of Monoacylglycerol Lipase (MAGL)

Inhibition of MAGL was studied as previously described[33] using commercial human recombinant MAGL. Briefly, the substrate 1,3-dihydroxypropan-2-yl 4-pyren-1-ylbutanoate was solubilized in HEPES-buffer containing Triton X-100 (0.2%) and EDTA (1 mM). The final substrate concentration was 100 μM. After incubation at 37 °C for 45 min, the enzyme reaction was terminated by addition of 200 μL acetonitrile/methanol (1:1, v/v) containing the internal standard 6-pyren-1-ylhexanoic acid. After centrifugation, the amount of 4-pyren-1-ylbutanoic acid released by the enzyme was determined in the supernatant by reversed-phase HPLC with fluorescence detection. In parallel, blank incubations in the absence of the enzyme were carried out and analyzed analogously. For the determination of the IC_{50} values, the ratios of the peak areas of enzyme product and internal standard obtained at different inhibitor concentrations, corrected by the corresponding value of the blank incubations, were used. The calculation of the IC_{50} values was carried out with the probit transformation.[42] The reference inhibitor ABX-1431 was purchased from Axon Medchem (Groningen, The Netherlands).

Inhibition of Butyrylcholinesterase (BuChE)

Inhibition of BuChE was determined as recently described[34] by measuring the inhibition of the release of benzoic acid from the substrate benzoylcholine using a commercially available enzyme isolated from horse serum (for details see Supporting Information).

Inhibition of Acetylcholinesterase (AChE)

Inhibition of AChE was determined by measuring the inhibition of the release of pyridin-2-ylmethanol from pyridin-2-ylmethyl acetate using a commercially available enzyme isolated from electric eel, as recently described (for details see Supporting Information).[34]

Inhibition Ccytosolic Phospholipase $A_2\alpha$ ($cPLA_2\alpha$)

Inhibition of $cPLA_2\alpha$ was determined in a similar manner as recently described[43] by measuring the inhibition of the release of arachidonic acid from 1-stearoyl-2-arachidonoyl-*sn*-glycero-3-phosphocholine in presence of the vesicle forming compound 1,2-dioleoyl-*sn*-glycerol. In deviation from this procedure, arachidonic acid was not quantified after on-line solid phase extraction by HPLC and UV-detection at 200 nm but directly by HPLC and single-quad MS-detection (for details see Supporting Information).

Determination of log P values

The partition coefficients (log P) were measured by reversed-phase HPLC according to a published OECD method as recently described.[24] Deviating from this, acetonitrile/PBS pH 7.4 (55:45, v/v) was used as mobile phase. PBS was prepared from phosphate

buffered saline tablets (one tablet dissolved in 200 mL of deionized water yielded 0.01 M phosphate buffer, 0.0027 M potassium chloride and 0.137 M sodium chloride, pH 7.4, at 25 °C) (Sigma-Aldrich, Steinheim, Germany).

Chemical Stability in Aqueous Solution

The determination was carried out as described.[24] Briefly, adapting the conditions of the FAAH assay the test compound was incubated in PBS buffer (pH 7.4) containing Triton X-100 (0.2%) and EDTA (1 mM) at 37 °C for 60 min. After centrifugation, the concentration of the compound was determined by reversed-phase HPLC with UV-detection at 220 nm. The relative amount of the test compound found in the aqueous sample after 60 min incubation at 37 °C was determined with the aid of a freshly prepared reference solution prepared analogously. Separation was achieved using a Synergi Polar-RP 80 Å column (4.6 mm (I.D.) × 150 mm, particle size 4 μm) (Phenomenex, Aschaffenburg, Germany) protected with a Phenomenex phenyl guard column (3 mm (I.D.) × 4 mm) or an Aqua C_{18} 125 Å column (4.6 mm (I.D.) × 150 mm, particle size 3 μm) (Phenomenex, Aschaffenburg, Germany) protected with a Phenomenex C_{18} guard column (3 mm (I.D.) × 4 mm).

Metabolic Stability in Porcine Liver S9 Fraction

The determination was carried out as recently reported.[24] Briefly, to a mixture of porcine liver S9 fraction (125 μL) and phosphate buffered saline (124 μL) was added a solution of the test compound (5 mM) in DMSO (1 μL). After incubation at 37 °C for 30 min, acetonitrile (500 μL) was added. The samples were cooled and centrifuged, and the supernatants were subjected to HPLC-MS analysis as described.[24] In parallel, controls were prepared by addition of the DMSO solution (5 mM) of the test compound (1 μL) to a mixture of porcine liver S9 fraction (125 μL), phosphate buffered saline (124 μL), and acetonitrile (500 μL). The controls were allowed to stand at room temperature for 30 min and further treated and analyzed as described above.

Metabolic Stability in Porcine Blood Plasma

The experiments were carried out as described for the determination of metabolic stability in porcine liver S9 fraction using 125 μL porcine plasma instead of 125 μL porcine liver homogenate each.[24]

Aqueous Solubility

Thermodynamic solubility was determined in the way previously described.[44] Briefly, to 1 mg of a test compound was added PBS (0.01 M, pH 7.4) (2 mL). The mixture was sonicated for 10 min in a bath sonifier and then shaken for 20 h at room temperature. After centrifugation at 12000 × g and 20 °C for 10 min, to an aliquot of the clear supernatant, acetonitrile was added, and the amount of the target compound present in the sample was determined by reversed-phase HPLC and UV detection at 240 nm. To exclude the presence of floated particles in the collected supernatant, two additional aliquots were taken from each centrifugation supernatant and treated and analyzed accordingly. With this method, for the reference indomethacin an aqueous solubility of 246 ± 18 μg/mL (mean ± standard deviation, n = 4) was determined.

Cellular Permeability

Permeability studies were carried out using a Caco-2 cell culture model. For cell culture routine, Caco-2 cells were cultivated using Dulbecco's Modified Eagle's Medium (DMEM) without phenol red, supplemented with 1% non-essential amino acids, 100 U/mL penicillin, 100 μg/mL streptomycin, 1% glutamine and 10% fetal bovine serum (FBS) in 10% CO_2 atmosphere at 37 °C (all materials were received from Biochrom AG, Berlin, Germany). Cells were split twice a week and used for experiments within passages 23–40. For the experiments, 50,000 cell/cm^2 were seeded on 12-well Transwell® polycarbonate membrane inserts (0.4 μm pores, 1.12 cm^2 cell growth area, Corning Inc., Tewksbury, USA). The cells were cultivated for 21 days to allow differentiation with a medium exchange every other day. Experimental setup was conducted using DMEM without FBS addition and monitoring of the trans-epithelial electrical resistance (TEER) using a cellZscope® device (nanoAnalytics GmbH, Münster, Germany). Filter with a TEER value below 150 Ω × cm^2 were not used for experiments. Incubation was carried out using 10 μM of the test compounds in the apical compartment in a total volume of 500 μL, while using 1000 μL in the basolateral compartment. After incubation of the Transwell™ plate at 37 °C for 2 h, 400 μL of the apical compartment solution was added to 20 μL aqueous EDTA-Na_2 solution (80 mM), and 800 μL of the basolateral compartment solution was added to 40 μL aqueous EDTA-Na_2 solution (80 mM), each placed in an Eppendorf safe-lock tube (1.5 mL) (final EDTA concentration: 3.8 mM). Subsequently, 100 μL of each mixture was diluted with 50 μl acetonitrile and transferred to an HPLC vial. The amount of test compound in the solutions of the apical and basolateral compartments was determined by HPLC-MS analysis using analogously prepared and treated reference solutions. 10 μL of each solution was injected into the HPLC-MS system, which was the same as described for the cPLA$_2\alpha$ inhibition assay (see Supporting Information). The autosampler temperature was 20 °C, column oven temperature was set to 30 °C. The elution was carried with a gradient program using acetonitrile/10 mM aqueous ammonium acetate (10:90), adjusted to pH 5 with formic acid (solvent A), and acetonitrile/10 mM aqueous ammonium acetate (90:10), adjusted to pH 5 with formic acid (solvent B), starting from 20% B and ending with 95% B. The flow rate was 0.3 mL/min and the chromatographic run time 30 min. The effluents were directed to the mass spectrometer by a divert valve from minute 2.5 to minute 24. The MS was operated in the positive SIM mode. The sum of the amounts of the test substance found in the apical and basolateral compartments after 2 h was set equal to the initial concentration of 10 μM. Equation (1) was used to calculate the general permeation coefficient, P, for which dc/dt = permeation rate, V = volume of acceptor compartment, c_0 = initial concentration in donor compartment, and A = area of the filter membrane.

$$P = \frac{dc}{dt} \times \frac{V}{A \times Co} \quad (1)$$

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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