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Design, Synthesis, and In Vitro Evaluation of Carbamate Derivatives of 2-Benzoxazolyl- and 2-Benzothiazolyl-(3-hydroxyphenyl)-methanones as Novel Fatty Acid Amide Hydrolase Inhibitors

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Fatty acid amide hydrolase (FAAH) is an intracellular serine hydrolase, which catalyzes the hydrolysis of the endocannabinoid *N*-arachidonylethanolamide to arachidonic acid and ethanolamine. FAAH also hydrolyzes another endocannabinoid, 2-arachidonoylglycerol (2-AG). However, 2-AG has been assumed to be hydrolyzed mainly by monoacylglycerol lipase (MAGL) or a MAGL-like enzyme. Inhibition of FAAH or MAGL activity might lead to beneficial effects in many physiological disorders such as pain, inflammation, and anxiety due to increased endocannabinoid-induced activation of cannabinoid receptors CB1 and CB2. In the present study, a total of 34 novel compounds were designed, synthesized, characterized, and tested against FAAH and MAGL-like enzyme activity. Altogether, 16 compounds were found to inhibit FAAH with half-maximal inhibition concentrations (IC₅₀) between 28 and 380 nM. All the active compounds belong to the structural family of carbamates. Compounds **14** and **18** were found to be the most potent FAAH inhibitors, which may serve as lead structures for novel FAAH inhibitors.

Introduction

Arachidonylethanolamide (AEA,^a Figure 1) and 2-arachidonoylglycerol (2-AG, Figure 1) are considered to be the most important endogenous agonists for the G protein-coupled cannabinoid receptors CB1 and CB2.^{1–3} CB1 receptors are predominantly located on presynaptic terminals in the central nervous system (CNS), whereas CB2 receptors are located mainly in peripheral tissues.^{4–6} However, it has been reported very recently that also CB2 receptors are expressed in the CNS.⁷ The endocannabinoids are inactivated rapidly by cellular reuptake followed by intracellular hydrolysis by specific enzymes.^{8,9} AEA is assumed to be transported into the cell by a specific transporter^{10–12} and rapidly hydrolyzed by the enzyme fatty acid amide hydrolase (FAAH).⁸ Also, like AEA, 2-AG is thought to be removed from its sites of action by cellular uptake and then hydrolyzed enzymatically. Although 2-AG can be hydrolyzed by FAAH,¹³ the main enzyme responsible for 2-AG hydrolysis in vivo is probably monoacylglycerol lipase¹⁴ (MAGL; EC 3.1.1.23) or MAGL-like enzyme.¹⁵ Due to the rapid inactivation, the cannabimimetic effects of the endocannabinoids remain very weak. The increase in the concentration of the endocannabinoids in the extracellular space can lead to several beneficial therapeutic effects¹⁶ such as treatment of pain¹⁷ and anxiety,¹⁸ reduction of intraocular pressure¹⁹ as well as increase of appetite.²⁰ The activation of CB2 receptors is involved in

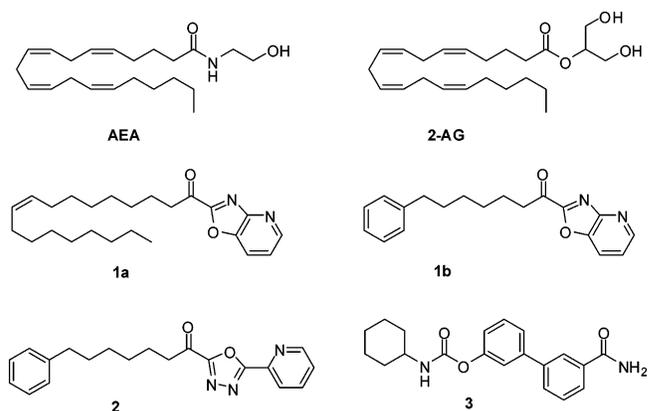


Figure 1. Structures of AEA, 2-AG, and some potent inhibitors of FAAH (**1a**, **1b**, **2**, and **3**).

the decrease of inflammation, lowering of blood pressure, and suppression of peripheral pain.²¹ Several potent synthetic CB receptor agonists as well as antagonists have been described (see for review Lambert and Fowler, 2005).²² In addition, inhibitors have been developed for the transport protein,^{12,23,24} although its existence is under debate.^{12,25,26} The enzyme inhibition could be a convenient way to elevate the endocannabinoid levels and thus increase the receptor activity.²⁷ Endocannabinoids are biosynthesized upon demand and released immediately from neurons afterward. By inhibiting FAAH or MAGL, the effect of endocannabinoids could be enhanced and more selective therapeutic effects achieved. Thus, the inhibition of these enzymes is of great interest to medicinal chemists nowadays.

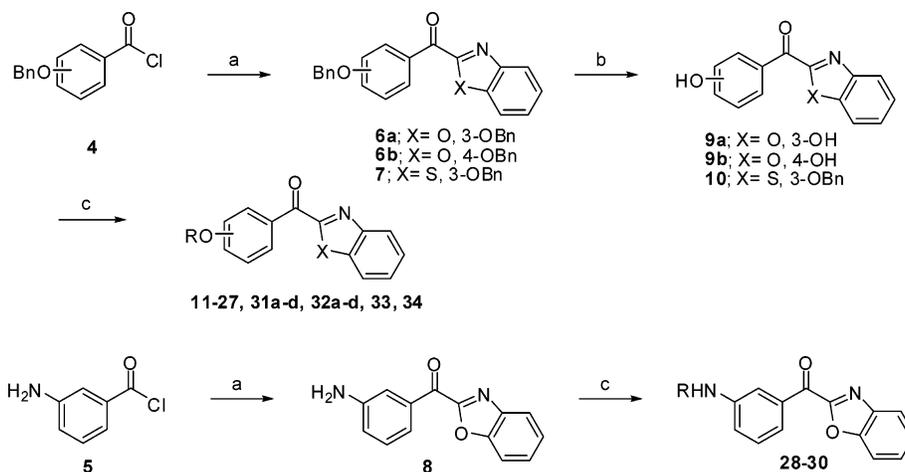
We have designed and synthesized a series of potential FAAH inhibitors and determined their inhibitory activities on FAAH and MAGL-like enzyme. We compared the structures of previously reported FAAH inhibitors and found that the most

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^a Abbreviations: FAAH, fatty acid amide hydrolase; MAGL, monoacylglycerol lipase; AEA, *N*-arachidonylethanolamine; 2-AG, 2-arachidonoylglycerol; CNS, central nervous system; IBX, *o*-iodoxybenzoic acid; DDQ, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone; MW, microwave irradiation; BSA, bovine serum albumin.

Scheme 1. Synthesis of Presented Compounds^a

^a Reagents and conditions: (a) benzoxazole or benzothiazole, *n*-BuLi, 1 M ZnCl₂/Et₂O, CuI, THF, -75 °C → 0 °C, 54–55%; (b) BF₃Et₂O, Me₂S, CH₂Cl₂, rt, 84–91% or *N*-1-BuPyrBr, MW, 30 s, 90%; (c) Et₃N or pyridine, RNCO, RCOCl or ROCOCl, toluene or CH₂Cl₂, rt or heating up to 93 °C, 41–99%.

potent compounds had very different structural moieties. Boger et al. (2000) have published potent and selective FAAH inhibitors that comprise of either 2-acyl-oxazolo[4,5-*b*]pyridines (**1a** and **1b**), 2-acyl-5-(2-pyridyl)-1,3,4-oxadiazoles (**2**), or with an alkyl tail (Figure 1).^{28,29} Additionally, the series of carbamates, including the highly active compound **3** (**URB597**),^{30,31} with promising pharmacological features have been developed.

Chemistry

Compound **1a** (Figure 1) was prepared as described by Boger et al.²⁸ The synthesis of the actual library was started from 3- or 4-hydroxybenzoic acid or 3-aminobenzoic acid. The phenol functionality was protected as benzyl ether and then the acids were converted to acid chlorides **4** and **5** (Scheme 1) according to literature procedures.^{32–34} Compound **4** was then coupled with benzoxazole or benzothiazole to prepare compounds **6a**, **6b**, and **7** following the method described by Harn et al.³⁵ Aniline **8** was prepared from **5** in a similar manner. Deprotection of the phenolic hydroxyl was first attempted with hydrogenation catalyzed by Pd/C. Unfortunately, under these conditions the methanone was reduced to alcohol before the cleavage of benzyl group. Although the alcohols were easily oxidized back to ketone with IBX³⁶ or DDQ,^{37,38} another method was needed to avoid the extra step. Finally, the removal of the benzyl protecting group without reducing the ketone was carried out using BF₃Et₂O and Me₂S.³⁹ Ionic liquid-based microwave-assisted cleavage of benzyl ether also proved to be as efficient a method.⁴⁰ Deprotected phenols **9a**, **9b**, **10**, and aniline **7** were then coupled with electrophiles (isocyanates, chloroformates, and acid chlorides; Scheme 1)

Results and Discussion

We have designed and prepared the novel series of FAAH inhibitors by combining the structural features of the known inhibitors presented in Figure 1. The carbamate and other carbonyl containing groups were attached to 2-methanone-benzothiazole and -benzoxazole via a phenylmethanone linker. The compounds were tested against FAAH and MAGL-like enzyme. From this series the most promising alkyl moiety was selected and other carbonyl derivatives were prepared. Additionally, two previously reported potent FAAH inhibitors, **1a** and **3** (see Figure 1), were also tested in our FAAH assay as

reference compounds. These results correlate with those reported in the literature.^{28–31} The structures of compounds and their inhibition potencies for FAAH and MAGL-like enzyme activity are presented in the Table 1.

The most potent FAAH inhibitor in this series was a cyclopentyl carbamate **14** (IC₅₀, 28 nM). Compounds **13**, **15**, **18**, **22**, **23**, and **26** inhibit FAAH with IC₅₀ values (32–56 nM) almost equal to that obtained for compound **14**. The inhibition of FAAH activity by these compounds was dependent on the carbamate group, as the compounds lacking this functionality were unable to inhibit FAAH even at 10 μM. This supports the mechanism by which carbamates like **3** inhibit FAAH by carbamylation of the enzyme's nucleophilic serine.⁴¹ Compounds **11**, **12**, and **13** as well as the corresponding sulfur analogs (**19**, **20**, and **21**) were found to have a trend for increasing potency for FAAH with increasing length of the alkyl group. It was noteworthy that compounds **18** and **26**, containing 3-methylbenzyl carbamate, had clearly higher inhibition activity against MAGL-like enzyme activity compared to the other compounds in this series. Thus, the introduction of the methyl group in the 3-position of the benzyl ring increased FAAH as well as MAGL-like enzyme inhibition. However, none of these compounds could inhibit MAGL-like activity at the nanomolar concentration range. Furthermore, there were no significant differences in FAAH or MAGL-like enzyme inhibition between the benzoxazoles and the benzothiazoles. Compounds **17** and **25** were not stable enough to give reliable inhibition activity. Apparently the carbamate was hydrolyzed.

To clarify the importance of the carbamate group, we prepared some other carbonyl derivatives for comparison. Carbonyl compounds **27** (ester), **28** (amide), **29** (urea), **30** (reverse carbamate), and **31a–d** and **32a–d** (carbonates) were not effective FAAH inhibitors. It was surprising that **30** did not inhibit FAAH or MAGL because its structural difference compared to compound **14** is only in the direction of the carbamate bond. Carbonates **31a–d** and **32a–d** were not stable enough in the assay conditions. The decomposition of compounds **17**, **25**, **31a–d**, and **32a–d** was detected with TLC (data not shown). 4-Substituted compounds **33** and **34** were also synthesized. The activity of these compounds against FAAH was still in nanomolar range (288 and 137 nM). It was noteworthy that a 10-fold difference was found between *meta*- (**14**, 28 nM) and *para*-substituted (**33**, 288 nM) cyclopentyl carbamates. In addition,

Table 1. IC₅₀ Values for the Inhibition of FAAH and MAGL-Like Enzymes Activity by Compounds Tested

Comp.	X	Y	R	IC ₅₀ (95% CI) ^a FAAH	MAGL	Comp.	X	Y	R	IC ₅₀ (95% CI) ^a FAAH	MAGL
6	O	3-O	Bn	No inh. at 10 μM	No inh. at 100 μM	25	S	3-O		nd ^c	nd ^c
11	O	3-O	CONHC ₂ H ₅	379 (303-474) nM	75% ^b	26	S	3-O		38 (33-43) nM	23 (20-27) μM ^d
12	O	3-O	CONH- <i>n</i> -C ₃ H ₇	109 (90-132) nM	57% ^b	27	O	3-O		88% ^e	No inh. at 100 μM
13	O	3-O	CONH- <i>n</i> -C ₄ H ₉	54 (46-64) nM	59% ^b	28	O	3-NH		95% ^e	No inh. at 100 μM
14	O	3-O		28 (23-34) nM	54% ^b	29	O	3-NH		94% ^e	No inh. at 100 μM
15	O	3-O		47 (36-62) nM	83% ^b	30	O	3-NH		95% ^e	No inh. at 100 μM
16	O	3-O		152 (122-189) nM	57% ^b	31a-d	O	3-O	a; Et b; <i>n</i> -Pr c; <i>n</i> -Bu d; Bn	nd ^c	nd ^c
17	O	3-O		nd ^c	nd ^c	32a-d	S	3-O	a; Et b; <i>n</i> -Pr c; <i>n</i> -Bu d; Bn	nd ^c	nd ^c
18	O	3-O		32 (26-40) nM	16 (14-18) μM ^d	33	O	4-O		288 (237-349) nM	14 (12-17) μM
7	S	3-O	Bn	No inh. at 10 μM	No inh. at 100 μM	34	O	4-O		137 (112-169) nM	25 (21-29) μM ^d
19	S	3-O	CONHC ₂ H ₅	238 (177-321) nM	80% ^b	3	-	-	Fig. 1	3.8 (2.9-5.0) nM lit. 4.6 nM ²⁵	No inh. at 100 μM ¹¹
20	S	3-O	CONH- <i>n</i> -C ₃ H ₇	143 (113-180) nM	70% ^b	1	-	-	Fig. 1	0.21 (0.16-0.28) nM lit. 40 nM, Ki; 0.23 nM ²⁴	No inh. at 100 μM
21	S	3-O	CONH- <i>n</i> -C ₄ H ₉	103 (81-131) nM	76% ^b						
22	S	3-O		47 (38-57) nM	71% ^b						
23	S	3-O		56 (41-77) nM	79% ^b						
24	S	3-O		121 (105-140) nM	75% ^b						

^a Values represent the mean of three independent experiments ($n = 3$) performed in duplicate (95% confidence intervals (95% CI) are given in parentheses).

^b Remaining enzyme activity (%) at 100 μM compound concentration ($n = 2$). ^c Not stable in used assay conditions. ^d Remaining enzyme activity at 1 mM was ~11–24%. ^e Remaining enzyme activity (%) at 10 μM compound concentration ($n = 2$).

compound **33** was found clearly more active against MAGL-like enzyme activity than **14**.

Conclusion

A series of carbonyl compounds as potential FAAH inhibitors were synthesized and evaluated for their FAAH and MAGL-like enzyme inhibition activity. Altogether, 16 carbamate containing compounds were found to inhibit FAAH with IC₅₀ values between 28 and 380 nM. These carbamate compounds **11–16**, **18–24**, **26**, **33**, and **34** were selective for FAAH as they were not able to inhibit MAGL-like enzyme at the high nanomolar concentration range. The most potent compounds for FAAH in this series containing a cyclopentyl carbamate (**14**) or 3-methyl-benzyl carbamate (**18**) group may serve as lead structures for novel FAAH inhibitors. Against MAGL-like enzyme activity, the best inhibitor was the *para*-substituted derivative of cyclopentyl carbamate (**33**) with 14 μM IC₅₀ value. These findings will further help designing more potent and selective inhibitors of FAAH and MAGL-like enzyme activity. It is also noteworthy that inhibiting both AEA- and 2-AG-hydrolyzing enzymes simultaneously through a combination of selective FAAH and MAGL inhibitors could lead to even more

beneficial therapeutic effects. This point of view calls for closer evaluation because the selectivity against other hydrolase enzymes might suffer.

Experimental Section

Chemistry. All solvents and reagents were obtained from commercial suppliers and used without further purification unless otherwise noted. Tetrahydrofuran (THF) was distilled from Na/benzophenone. All dry reactions were performed under argon in flame-dried glassware. Analytical thin-layer chromatography was carried out on Merck silica gel F₂₅₄ (60 Å, 40–63 μm, 230–400 mesh) precoated aluminum sheets and detected under UV light. Silica gel (230–400 mesh) for column chromatography was purchased from Merck. Melting points (mp) were determined in open capillaries using a Gallenkamp melting point apparatus and are uncorrected. Nuclear magnetic resonance (¹H NMR and ¹³C NMR) spectra were recorded on a Bruker Avance DPX 400 spectrometer operating at 400 MHz for ¹H and 100 MHz for ¹³C. Chemical shifts are reported in ppm on the δ scale from an internal standard of residual solvent (CDCl₃ 7.26 and 77.0 ppm; DMSO-*d*₆ 2.50 and 39.52 ppm). Coupling constants (*J*) are reported in Hz. Infrared (IR) spectra were recorded using a Perkin-Elmer Spectrum One FT-IR spectrometer, and values are reported as frequency (ν)

and expressed in cm^{-1} . Elemental analyses were recorded on a Perkin-Elmer 2400 CHN.

General Procedure for the Preparation of 6a, 6b, 7, and 8 from Acid Chlorides 4 and 5; Benzo[d]oxazol-2-yl(3-(benzyloxy)phenyl)methanone (6a). To a solution of benzoxazole (727 mg, 6.1 mmol, 100 mol %) in THF (35 mL) was added dropwise *n*-BuLi (1.8 M in hexane, 3.7 mL, 6.7 mmol, 110 mol %) at -75°C over a period of 10 min. After 30 min, ZnCl_2 (1.66 g, 12.2 mmol, 200 mol %) in Et_2O (20 mL) was added. The mixture was warmed to 0°C , and after 45 min, CuI (1.16 mg, 6.1 mmol, 100 mol %) was added. After another 10 min, acid chloride **4** (1.5 g, 6.1 mmol, 100 mol %) in THF (10 mL) was added. The mixture was stirred at 0°C for another 45 min, diluted with EtOAc (400 mL), and washed successively with 1:1 $\text{H}_2\text{O}/25\%$ aq ammonia (100 mL), H_2O (100 mL), and brine (100 mL). The organic phase was dried over anhydrous Na_2SO_4 , filtered, and evaporated to yield the crude product as a tan solid, which was purified with flash chromatography (5% EtOAc in hexane) and recrystallized (EtOAc/hexane) to give **6a** (1.08 g, 54%) as a yellow solid: mp $95\text{--}97^\circ\text{C}$; R_f (20% EtOAc in hexane) 0.50; $^1\text{H NMR}$ (CDCl_3) δ 8.22 (d, 1H, $J = 7.7$ Hz), 8.13 (dd, 1H, $J = 2.4, 1.6$ Hz), 7.93 (dd, 1H, $J = 7.9$ Hz), 7.70 (d, 1H, $J = 8.2$ Hz), 7.54 (td, 1H, $J = 7.8, 1.0$ Hz), 7.49–7.44 (m, 4H), 7.41–7.28 (m, 4H), 5.17 (s, 2H); $^{13}\text{C NMR}$ (CDCl_3) δ 180.1, 158.8, 157.1, 150.4, 140.7, 136.4, 136.2, 129.7, 128.6, 128.4, 128.1, 127.6, 125.7, 124.1, 122.4, 121.7, 116.0, 111.8, 70.3; IR (KBr) 3030, 2861, 1648, 1604; Anal. ($\text{C}_{21}\text{H}_{15}\text{NO}_3$) C, H, N.

General Procedure for the Deprotection of 6a, 6b, and 7 to Prepare 9a, 9b, and 10. Benzo[d]oxazol-2-yl(3-hydroxyphenyl)methanone (9a). Compound **6a** (5.0 g, 15.2 mmol, 100 mol %) was stirred in a solution of boron trifluoride diethyl etherate (6.9 mL, 55 mmol, 360 mol %) and dimethylsulfide (10 mL, 136 mmol, 900 mol %) in dry CH_2Cl_2 (100 mL) at room temperature for 72 h. The mixture was then quenched with H_2O (120 mL) and diluted with CH_2Cl_2 (350 mL). The organic phase was washed with brine (2×100 mL), dried over anhydrous Na_2SO_4 , filtered, and evaporated to yield a crude product as a red solid, which was recrystallized (EtOAc/hexane) to yield **9a** (3.06 g, 84%) as light yellow crystals: mp $125\text{--}128^\circ\text{C}$; R_f (50% EtOAc/hexane) 0.61; $^1\text{H NMR}$ (CDCl_3) δ 8.17 (ddd, 1H, $J = 7.8, 1.6, 1.0$ Hz), 8.04 (dd, 1H, $J = 2.4, 1.6$ Hz), 7.94 (ddd, 1H, $J = 8.0, 1.3, 0.7$ Hz), 7.69 (dt, 1H, $J = 8.2, 0.9$ Hz), 7.57–7.52 (m, 1H), 7.48–7.41 (m, 2H), 7.19 (ddd, 1H, $J = 8.1, 2.7, 0.9$ Hz), 5.74 (s, 1H); $^{13}\text{C NMR}$ (CDCl_3) δ 180.2, 157.1, 156.0, 150.4, 140.6, 136.2, 130.0, 128.5, 125.8, 123.7, 122.3, 121.9, 117.2, 111.9; IR (KBr) 3463, 1651, 1593, 1525; Anal. ($\text{C}_{14}\text{H}_9\text{NO}_3$) C, H, N.

Benzo[d]oxazol-2-yl(4-hydroxyphenyl)methanone (9b). Compound **6b** (510 mg, 1.55 mmol, 100 mol %) and *N*-butylpyridinium bromide (820 mg, 3.8 mmol, 245 mol %) were added to a 10 mL CEM reaction tube, closed with a septum, and irradiated with CEM microwave apparatus for 30 s (power 125 W, $T_{\text{max}} = 125^\circ\text{C}$). The reaction mixture was dissolved in EtOAc (70 mL), washed with water (50 mL) and brine (50 mL), dried over Na_2SO_4 , filtered, and evaporated. The yellow solid was then purified with flash chromatography (50% EtOAc in hexane) and recrystallization (EtOAc/hexane) giving **9b** (310 mg, 86%) as a yellow solid: mp $188\text{--}189^\circ\text{C}$; R_f (50% EtOAc in hexane) 0.60; $^1\text{H NMR}$ ($\text{DMSO-}d_6$) δ 10.80 (s, 1H), 8.45–8.42 (m, 2H), 8.04 (d, 1H, $J = 7.9$ Hz), 7.93 (d, 1H, $J = 8.2$ Hz), 7.66–7.62 (m, 1H), 7.57–7.53 (m, 1H), 7.02–6.99 (m, 2H); $^{13}\text{C NMR}$ ($\text{DMSO-}d_6$) δ 177.9, 163.6, 157.2, 149.7, 140.1, 133.6, 128.3, 126.1, 125.8, 121.9, 115.5, 111.9; IR (KBr) 3254, 3050, 1599, 1579; Anal. ($\text{C}_{14}\text{H}_9\text{NO}_3$) C, H, N.

General Procedure for the Preparation of Phenolic Carbamates 11–26, 33, and 34 from 9a, 9b, and 10. 3-(Benzo[d]oxazole-2-carbonyl)phenyl ethylcarbamate (11). To a solution of benzo[d]oxazol-2-yl(3-hydroxyphenyl)methanone (100 mg, 0.42 mmol, 100 mol %) in dry toluene (4 mL) were added triethylamine (60 μL , 0.42 mmol, 100 mol %) and ethyl isocyanate (166 μL , 2.1 mmol, 500 mol %). After stirring at room temperature for 12 h, the reaction was complete, as judged by TLC (5% Et_2O in CH_2Cl_2), and the mixture was diluted with EtOAc (8 mL), filtered through a pad of silica gel, and evaporated to dryness. Recrystal-

lization from EtOAc/hexane yielded **11** (121 mg, 93%) as a white cotton-like solid: mp $141\text{--}142^\circ\text{C}$; R_f (5% Et_2O in CH_2Cl_2) 0.70; $^1\text{H NMR}$ (CDCl_3) δ 8.46 (d, 1H, $J = 7.7$ Hz), 8.32 (s, 1H), 7.94 (d, 1H, $J = 8.1$ Hz), 7.70 (d, 1H, $J = 8.2$ Hz), 7.57–7.45 (m, 4H), 5.19 (br s, 1H), 3.33 (qui, 2H, $J = 6.8$ Hz), 1.23 (t, 3H, $J = 7.2$ Hz); $^{13}\text{C NMR}$ (CDCl_3) δ 179.4, 156.9, 154.0, 151.2, 150.4, 140.7, 136.0, 129.4, 128.5, 127.9, 127.8, 125.7, 123.9, 122.4, 111.8, 36.2, 15.0; IR (KBr) 3345, 2976, 1712, 1662, 1527; Anal. ($\text{C}_{17}\text{H}_{14}\text{N}_2\text{O}_4$) C, H, N.

3-(Benzo[d]oxazole-2-carbonyl)phenyl cyclopentanecarboxylate (27). A mixture of **6a** (130 mg, 0.54 mmol, 100 mol %), cyclopentanoyl chloride (86 mg, 0.65 mmol, 120 mol %), and TEA (76 μL , 0.54 mmol, 100 mol %) was stirred in dry toluene (6 mL) for 20 min at room temperature, then quenched with ice (5 mL) and satd NaHCO_3 (5 mL), and extracted with EtOAc (2×25 mL). The combined organic phase was washed with brine (20 mL), dried (Na_2SO_4), filtered, and evaporated. Recrystallization from EtOAc/hexane gave **27** (169 mg, 93%) as white crystals: mp $147\text{--}148^\circ\text{C}$; R_f (5% MeOH in CH_2Cl_2) 0.90; $^1\text{H NMR}$ (CDCl_3) δ 8.53 (ddd, 1H, $J = 7.8, 1.5, 1.1$ Hz), 8.27–8.25 (m, 1H), 7.96 (td, 1H, $J = 8.0, 0.7$ Hz), 7.74–7.70 (m, 1H), 7.61–7.54 (m, 2H), 7.49 (ddd, 1H, $J = 8.4, 7.4, 1.1$ Hz), 7.43 (ddd, 1H, $J = 8.1, 2.4, 1.0$ Hz), 3.00–3.09 (m, 1H), 2.11–1.95 (m, 4H), 1.85–1.75 (m, 2H), 1.73–1.64 (m, 2H); $^{13}\text{C NMR}$ (CDCl_3) δ 179.4, 175.0, 156.8, 151.0, 150.4, 140.7, 136.2, 129.6, 128.6, 128.5, 127.8, 125.8, 123.9, 122.5, 111.9, 43.8, 30.1, 25.9; IR (KBr) 2971, 2872, 1750, 1661, 1531; Anal. ($\text{C}_{20}\text{H}_{17}\text{NO}_4$) C, H, N.

***N*-(3-(Benzo[d]oxazole-2-carbonyl)phenyl)cyclopentanecarboxamide (28).** To a solution of **8** (71 mg, 0.30 mmol, 100 mol %) in dry CH_2Cl_2 (3.2 mL) was added cyclopentanoyl chloride (54 mg, 0.41 mmol, 136 mol %). An immediate reaction formed a thick suspension that was quenched with ice and partitioned between CH_2Cl_2 (20 mL) and water (10 mL). The organic layer was washed with satd NaHCO_3 (2×10 mL) and brine (10 mL), dried over Na_2SO_4 , and evaporated. Recrystallization from 30% EtOAc in hexane yielded **28** (61 mg, 61%) as white crystals: mp $180\text{--}182^\circ\text{C}$; R_f (50% EtOAc in hexane) 0.51; $^1\text{H NMR}$ ($\text{DMSO-}d_6$) δ 10.20 (s, 1H), 8.56 (t, 1H, $J = 1.8$ Hz), 8.22–8.18 (m, 1H), 8.07–8.02 (m, 2H), 7.97–7.94 (m, 1H), 7.69–7.64 (m, 1H), 7.59–7.54 (m, 2H), 2.83 (m, 1H), 1.88 (m, 2H), 1.73 (m, 4H), 1.57 (m, 2H); $^{13}\text{C NMR}$ ($\text{DMSO-}d_6$) δ 179.9, 174.8, 156.9, 149.8, 140.2, 139.8, 135.2, 129.0, 128.7, 126.0, 125.7, 124.7, 122.1, 120.4, 112.0, 45.3, 30.1, 25.7; IR (KBr) 3289, 2960, 1656, 1603, 1536; Anal. ($\text{C}_{20}\text{H}_{18}\text{N}_2\text{O}_3$) C, H, N.

1-(3-(Benzo[d]oxazole-2-carbonyl)phenyl)-3-cyclopentylurea (29). To a solution of **8** (71 mg, 0.30 mmol, 100 mol %) in dry CH_2Cl_2 (3.2 mL) was added cyclopentyl isocyanate (88 μL , 0.76 mmol, 250 mol %). After stirring at room temperature for 16 h, another portion of cyclopentyl isocyanate (88 μL , 0.76 mmol, 250 mol %) was added, and the mixture was refluxed for another 24 h. The mixture was diluted with EtOAc (10 mL), washed with H_2O (5 mL) and brine (5 mL), dried over Na_2SO_4 , filtered, and evaporated. Purification with flash chromatography (2% MeOH in CH_2Cl_2) and recrystallization from MeOH (5 mL) yielded **29** (58 mg, 55%) as off-white crystals: mp $216\text{--}217^\circ\text{C}$; R_f (5% MeOH in CH_2Cl_2) 0.35; $^1\text{H NMR}$ ($\text{DMSO-}d_6$) δ 8.62 (s, 1H), 8.34 (t, 1H, $J = 1.9$ Hz), 8.06–8.00 (m, 2H), 7.95 (app d, 1H, $J = 8.2$ Hz), 7.84–7.80 (m, 1H), 7.69–7.64 (m, 1H), 7.49 (t, 1H, $J = 7.9$ Hz), 6.25 (d, 1H, $J = 7.2$ Hz), 3.96 (sext, 1H, $J = 6.7$ Hz), 1.90–1.81 (m, 2H), 1.70–1.49 (m, 4H), 1.43–1.34 (m, 2H); $^{13}\text{C NMR}$ ($\text{DMSO-}d_6$) δ 180.1, 157.0, 154.7, 149.8, 140.9, 140.1, 135.2, 129.0, 128.6, 125.9, 123.6, 123.3, 122.1, 118.9, 112.0, 50.9, 32.8, 23.1; IR (KBr) 3335, 2956, 2925, 2857, 1666, 1646, 1626, 1564; Anal. ($\text{C}_{20}\text{H}_{19}\text{N}_3\text{O}_3$) C, H, N.

Cyclopentyl 3-(Benzo[d]oxazole-2-carbonyl)phenylcarbamate (30). A solution of triphosgene (353 mg, 1.19 mmol, 435 mol %), cyclopentanol (330 μL , 3.60 mmol, 1300 mol %), and pyridine (250 μL , 3.09 mmol, 1100 mol %) in dry CH_2Cl_2 (1.2 mL) was stirred in an ice bath for 2 h. Compound **8** (65 mg, 0.273 mmol, 100 mol %) dissolved in dry CH_2Cl_2 (4 mL) was added to the mixture. The mixture was stirred for an hour and quenched with

ice and extracted with CH_2Cl_2 (10 mL). The organic phase was washed with water (5 mL) and brine (5 mL), dried over Na_2SO_4 , filtered, and evaporated. Recrystallization with EtOAc/hexane yielded **30** (50 mg, 52%) as off-white solid: mp 140–141 °C; R_f (35% EtOAc in hexane) 0.49; $^1\text{H NMR}$ (CDCl_3) δ 8.39 (t, 1H, $J = 1.9$ Hz), 8.28–8.24 (m, 1H), 7.98–7.89 (m, 2H), 7.72 (d, 1H, $J = 8.2$ Hz), 7.59–7.45 (m, 3H), 6.76 (br s, 1H), 5.27–5.21 (m, 1H), 1.96–1.86 (m, 2H), 1.84–1.70 (m, 4H), 1.68–1.58 (m, 2H); $^{13}\text{C NMR}$ (CDCl_3) δ 180.0, 157.0, 153.3, 150.4, 140.7, 138.6, 135.6, 129.5, 128.5, 125.8, 125.7, 124.3, 122.4, 120.4, 111.9, 78.5, 32.8, 23.6; IR (KBr) 3303, 2961, 1701, 1663, 1591, 1528; Anal. ($\text{C}_{20}\text{H}_{18}\text{N}_2\text{O}_4$) C, H, N.

General Procedure for the Preparation of Phenolic Carbonates from 9 and 10. **3-(Benzo[d]oxazole-2-carbonyl)phenyl Ethyl Carbonate (31a).** To a solution of compound **9** (100 mg, 0.42 mmol, 100 mol %) in dry toluene (4 mL) was added triethylamine (70 μL , 0.50 mmol, 120 mol %) and ethyl chloroformate (48 μL , 0.50 mmol, 120 mol %). The yellow color of the mixture disappeared immediately after the addition of chloroformate. After stirring at room temperature for 5 min, the reaction was complete (TLC, 5% Et_2O in CH_2Cl_2), and the mixture was evaporated to dryness. Recrystallization from EtOAc/hexane yielded **31a** (113 mg, 86%) as a white solid: mp 116–117 °C; R_f (5% Et_2O in CH_2Cl_2) 0.50; $^1\text{H NMR}$ (CDCl_3) δ 8.51 (dt, 1H, $J = 7.9, 1.4$ Hz), 8.44 (t, 1H, $J = 1.8$ Hz), 7.96 (d, 1H, $J = 8.1$ Hz), 7.72 (d, 1H, $J = 8.2$ Hz), 7.62–7.46 (m, 4H), 4.46 (q, 2H, $J = 7.1$ Hz), 1.42 (t, 3H, $J = 7.1$ Hz); $^{13}\text{C NMR}$ (CDCl_3) δ 179.1, 156.8, 153.3, 151.2, 150.5, 140.7, 136.3, 129.7, 128.6, 128.6, 127.1, 125.8, 123.6, 122.5, 111.9, 65.2, 14.2; IR (KBr) 1762, 1667, 1582, 1526; Anal. ($\text{C}_{17}\text{H}_{13}\text{NO}_5$) C, H, N.

Animals and Preparation of Rat Brain Homogenate for FAAH Assay. Eight-week-old male Wistar rats were used in these studies. All animal experiments were approved by the local ethics committee. The animals lived in a 12 h light/12 h dark cycle (lights on at 0700 h), with water and food available ad libitum.

The rats were decapitated, and whole brains minus cerebellum were dissected and homogenized in one volume (v/w) of ice-cold 0.1 M potassium phosphate buffer (pH 7.4) with a Potter–Elvehjem homogenizer (Heidolph). The homogenate was centrifuged at 10 000 g for 20 min at 4 °C, and the resulting supernatant was used as a source of FAAH activity. The protein concentration of the supernatant (7.2 mg/mL) was determined by the method of Bradford with BSA as a standard.⁴² Aliquots of the supernatant were stored at –80 °C until use.

Animals and Preparation of Rat Cerebellar Membranes for MGL Assay. Four-week-old male Wistar rats were used in these studies. All animal experiments were approved by the local ethics committee. The animals lived in a 12 h light/12 h dark cycle (lights on at 0700 h), with water and food available ad libitum. The rats were decapitated, 8 h after lights on (1500 h), whole brains were removed, dipped in isopentane on dry ice, and stored at –80 °C. Membranes were prepared as previously described.^{43–45}

Briefly, cerebella (minus brain stem) from eight animals were weighed and homogenized in nine volumes of ice-cold 0.32 M sucrose with a glass Teflon homogenizer. The crude homogenate was centrifuged at low speed (1000 $\times g$ for 10 min at 4 °C) and the pellet was discharged. The supernatant was centrifuged at high speed (100 000 $\times g$ for 10 min at 4 °C). The pellet was resuspended in ice-cold deionized water and washed twice, repeating the high-speed centrifugation. Finally, membranes were resuspended in 50 mM Tris-HCl, pH 7.4, with 1 mM EDTA and aliquoted for storage at –80 °C. The protein concentration of the final preparation, measured by the Bradford method,⁴² was 11 mg mL^{-1} .

In Vitro Assay for FAAH Activity. The endpoint enzymatic assay was developed to quantify FAAH activity with tritium-labeled arachidonylethanolamide [ethanolamine $1\text{-}^3\text{H}$]. The assay buffer was 0.1 M potassium phosphate (pH 7.4) used and test compounds were dissolved in DMSO (the final DMSO concentration was max 5% v/v). The incubations were performed in the presence of 0.5% (w/v) BSA (essentially fatty acid free). Test compounds were preincubated with rat brain homogenate protein (18 μg) for 10 min

at 37 °C (60 μL). At the 10 min time point, arachidonylethanolamide was added so that its final concentration was 2 μM (containing $50 \times 10^{-3} \mu\text{Ci}$ of 60 Ci/mmol [^3H]AEA) and the final incubation volume was 100 μL . The incubations proceeded for 10 min at 37 °C. Ethyl acetate (400 μL) was added at the 20 min time point to stop the enzymatic reaction. Additionally, 100 μL of unlabeled ethanolamine (1 mM) was added as a “carrier” for radioactive ethanolamine. Samples were centrifuged at 16 000 g for 4 min at room temperature, and aliquots (100 μL) from aqueous phase containing [ethanolamine $1\text{-}^3\text{H}$] were measured for radioactivity by liquid scintillation counting (Wallac 1450 MicroBeta; Wallac Oy, Finland).

In Vitro Assay for MGL Activity. The assay for MGL has been described previously.⁴⁶ Briefly, experiments were carried out with preincubations (80 μL , 30 min at 25 °C) containing 10 μg membrane protein, 44 mM Tris-HCl (pH 7.4), 0.9 mM EDTA, 0.5% (wt/vol) BSA, and 1.25% (vol/vol) DMSO as a solvent for inhibitors. The preincubated membranes were kept at 0 °C just prior to the experiments. The incubations (90 min at 25 °C) were initiated by adding 40 μL of preincubated membrane cocktail, in a final volume of 400 μL . The final volume contained 5 μg membrane protein, 54 mM Tris-HCl (pH 7.4), 1.1 mM EDTA, 100 mM NaCl, 5 mM MgCl_2 , 0.5% (wt/vol) BSA, and 50 μM of **1**. At time points of 0 and 90 min, 100 μL samples were removed from the incubation, acetonitrile (200 μL) was added to stop the enzymatic reaction, and the pH of the samples was simultaneously decreased to 3.0 with phosphoric acid (added to acetonitrile) to stabilize compound **1** against acyl migration to 1(3)-AG. Samples were centrifuged at 23 700 g for 4 min at room temperature prior to HPLC analysis of the supernatant.

HPLC Method. The analytical HPLC was performed as previously described.¹⁵ Briefly, the analytical HPLC system consisted of a Merck Hitachi (Hitachi Ltd., Tokyo, Japan) L-7100 pump, D-7000 interface module, L-7455 diode-array UV detector (190–800 nm, set at 211 nm), and L-7250 programmable autosampler. The separations were accomplished on a Zorbax SB-C18 endcapped reversed-phase precolumn (4.6 \times 12.5 mm, 5 μm) and column (4.6 \times 150 mm, 5 μm ; Agilent). The injection volume was 50 μL . A mobile phase mixture of 28% phosphate buffer (30 mM, pH 3.0) in acetonitrile was used at a flow rate of 2.0 mL min^{-1} . Retention times were 5.8 min for **1**, 6.3 min for 1(3)-AG, and 10.2 min for arachidonic acid. The relative concentrations of **1**, 1(3)-AG, and arachidonic acid were determined by the corresponding peak areas. This was justified by the equivalence of response factors for the studied compounds and was supported by the observation that the sum of the peak areas was constant throughout the experiments.

Data Analyses. The results from the enzyme inhibition experiments are presented as mean \pm 95% confidence intervals of at least three independent experiments performed in duplicate. Data analyses for the dose–response curves were calculated as nonlinear regressions using GraphPad Prism 4.0 for Windows.

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Supporting Information Available: Spectroscopic characterization and elemental analysis data for all novel compounds. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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