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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

Mikko J. Myllymäki,† Susanna M. Saario,‡ Antti O. Kataja,† Joel A. Castillo-Melendez,† Tapio Nevalainen,‡ Risto O. Juvonen,§ Tomi Järvinen,‡ and Ari M. P. Koskinen*,†

Laboratory of Organic Chemistry, Department of Chemical Technology, Helsinki University of Technology, Post Office Box 6100, Kemistintie 1, FIN-02015 TKK, Finland, and Department of Pharmaceutical Chemistry and Department of Pharmacology and Toxicology, University of Kuopio, Post Office Box 1627, FIN-70211 Kuopio, Finland

Fatty acid amide hydrolase (FAAH) is an intracellular serine hydrolase, which catalyzes the hydrolysis of the endocannabinoid N-arachidonoylthanolamide 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

Arachidonoylthanolamide (AEA, 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 transporter10–12 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 lipase (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 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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1 Helsinki University of Technology.
2 Department of Pharmaceutical Chemistry, University of Kuopio.
3 Department of Pharmacology and Toxicology, University of Kuopio.

Abbreviations: FAAH, fatty acid amide hydrolase; MAGL, monoacylglycerol lipase; AEA, N-arachidonoylthanolamine; 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.
potent compounds had very different structural moieties. Boger et al. (2000) have published potent and selective FAAH inhibitors that comprise of 2-acyl-oxazol[4,5-b]pyridines (1a and 1b), 2-acyl-5-(2-pyridyl)-1,3,4-oxadiazoles (2), or with an alkyl tail (Figure 1). Additionally, the series 1a to literature procedures. 32 Additionally, two previously reported potent FAAH inhibitors, 1a and other carbonyl derivatives were prepared. Additionally, two previously reported potent FAAH inhibitors, 1a and 3 (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.31 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 (carbamates) 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 carbanates. In addition,
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-d6 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-benzoxylphenyl)methanone (6a).** To a solution of benzoazole (727 mg, 6.1 mmol, 100 %) in THF (35 mL) was added dropwise n-BuLi (1.8 M in hexane, 3.7 mL, 6.7 mmol, 110 %) at −75 °C over a period of 10 min. After 30 min, ZnCl₂ (1.66 g, 12.2 mmol, 200 mol %) in Et₂O (20 mL) was added. The mixture was warmed to 0 °C, and after 45 min, Cul (1.16 mg, 6.1 mmol, 100 mol %) was added. After another 10 min, acid chloride 4 (1.5 g, 6.1 mmol, 100 %) 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 H₂O/25% aq ammonia (100 mL), H₂O (100 mL), and brine (100 mL). The organic phase was dried over anhydrous Na₂SO₄, 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 95–97 °C; Rₚ (20 % EtOAc in hexane) 0.50; ¹H NMR (CDCl₃) 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); ¹³C NMR (CDCl₃) 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, 124.7, 116.0, 111.8, 70.3; IR (KBr) 3030, 2861, 1648, 1604; Anal. (C₁₂H₁₀N₂O₂) 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 CH₂Cl₂ (100 mL) at room temperature for 72 h. The mixture was then quenched with H₂O (120 mL) and diluted with CH₂Cl₂ (350 mL). The organic phase was washed with brine (2 × 100 mL), dried over anhydrous Na₂SO₄, 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 125–128 °C; Rₚ (50 % EtOAc in hexane) 0.61; ¹H NMR (CDCl₃) δ 8.17 (dd, 1H, J = 7.8, 1.6, 1.0 Hz), 8.04 (dd, 1H, J = 2.4, 1.6 Hz), 7.94 (dd, 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 (dd, 1H, J = 8.1, 2.7, 0.9 Hz), 5.74 (s, 1H); ¹³C NMR (CDCl₃) δ 180.2, 157.1, 156.0, 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. (C₁₀H₈N₂O₂) C, H, N.

**Benzo[d]oxazol-2-yl(4-hydroxyphenyl)methanone (9b).** Compound 6b (510 mg, 1.55 mmol, 100 mol %) and 2-n-butylpyridinium bromide (820 mg, 3.8 mmol, 245 mol %) were added to a 10 mL reaction tube, closed with a septum, and irradiated with CEM bromide (820 mg, 3.8 mmol, 245 mol %) were added to a 10 mL reaction mixture and expressed in cm

**Cyclization from EtoAc/hexane.**
ice and extracted with CH₂Cl₂ (10 mL). The organic phase was washed with water (5 mL) and brine (5 mL), dried over Na₂SO₄, filtered, and evaporated. Recrystallization with EtOAc/hexane yielded 30 (50 mg, 52%) as off-white solid: mp 140–141 °C; R₉ (35% EtOAc in hexane) 0.49; [α]H NMR (CDCl₃) 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); ¹³C NMR (CDCl₃) δ 180.0, 157.0, 153.3, 150.4, 147.0, 138.6, 135.6, 129.5, 128.5, 125.8, 124.3, 122.4, 111.9, 78.5, 32.8, 23.6; IR (KBr) 3303, 2961, 1701, 1663, 1591, 1528; Anal. (C₂H₃N₂O₄) C, H, N.

**General Procedure for the Preparation of Phenolic Carbonamide from 9 and 11.** A solution of compound 9 (100 mg, 0.42 mmol, 100 mol %) and ethyl chloroformate (48 µL, 0.50 mmol, 120 mol %) in dry toluene (4 mL) was added triethylamine (70 µL, 0.50 mmol, 120 mol %) and ethyl chloroforomate (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% Et₂O in CH₂Cl₂), and the mixture was evaporated to dryness. Recrystallization from EtOAc/hexane yielded 31a (113 mg, 86%) as a white solid: mp 116–117 °C; R₉ (5% EtOAc in CH₂Cl₂) 0.50; [α]H NMR (CDCl₃) 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). ¹³C NMR (CDCl₃) 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, 119.8, 68.2; 1H NMR (CDCl₃) δ 8.24 (m, 1H), 7.98 (d, 1H, J = 3.3 Hz), 7.89 (m, 2H), 7.72 (d, 1H, J = 6.9 Hz); 13C NMR (CDCl₃) 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, 119.8, 68.2, 14.2; IR (KBr) 1762, 1667, 1582, 1526; Anal. (C₆H₅N₂O₄) 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 decapitated 8 h after lights on (1500 h), whole brains were homogenized in one volume (v/w) of ice-cold 0.32 M K₂HPO₄ (pH 7.4), with a Potter–Elvehjem homogenizer (Heidolph). The homogenate was centrifuged at 23 700 g for 10 min at 4 °C. The protein concentration of the final preparation, measured by the Bradford method, 42 was 11 mg mL⁻¹. The pellets 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 × g for 10 min at 4 °C) and the pellet was discharged. The supernatant was centrifuged at high speed (100 000 × 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, 43,44 was 11 mg mL⁻¹.

**In Vitro Assay for FAAH Activity.** The endpoint enzymatic assay was developed to quantify FAAH activity with tritium-labeled arachidonoylthanolamide [ethanolamine 1-³H] 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 1-³H] 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 50 µM of I. 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. 47,48 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 × 12.5 mm, 5 µm) and column (4.6 × 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 I, 6.3 min for 1(3)-AG, and 10.2 min for arachidonic acid. The relative concentrations of I, 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 ± 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.

**References**


