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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 $N$-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 (IC$_{50}$) 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 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 effects such as treatment of pain16 and anxiety,17 reduction of intraocular pressure18 as well as increase of appetite.19 The activation of CB2 receptors is involved in the decrease of inflammation, lowering of blood pressure, and suppression of peripheral pain.20 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
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). Additionally, two previously reported potent FAAH inhibitors, 1a and 32b, were also tested in our FAAH assay as reference compounds. These results correlate with those reported in the literature.30–31 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 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,
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 IC_{50} 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 IC_{50} 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 (1 H NMR and 13 C NMR) spectra were recorded on a Bruker Avance DPX 400 spectrometer operating at 400 MHz for 1 H and 100 MHz for 13 C. 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-(benzoxoly)-phenylnethanone (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\(_2\)O (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 H\(_2\)O:25%aq ammonia (100 mL), H\(_2\)O (100 mL), and brine (100 mL). The organic phase was dried over anhydrous Na\(_2\)SO\(_4\), 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 95–97 °C; \(R_f\) (20% EtOAc in hexane) 0.50; \(^1\)H NMR (CDCl\(_3\)) \(\delta\): 8.22 (d, 1 H, \(J = 7.7 \text{ Hz}\)), 8.13 (dd, 1 H, \(J = 2.4, 1.6 \text{ Hz}\)), 7.93 (dd, 1 H, \(J = 7.9 \text{ Hz}\)), 7.70 (d, 1 H, \(J = 8.2 \text{ Hz}\)), 7.54 (td, 1 H, \(J = 7.8, 1.0 \text{ Hz}\)), 7.49–7.44 (m, 4H), 7.41–7.28 (m, 4H), 5.17 (s, 2H); \(^13\)C NMR (CDCl\(_3\)) \(\delta\): 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, 122.1, 116.0, 111.8, 70.3; IR (KBr) 3030, 2861, 1648, 1604; Anal. (C\(_{21}\)H\(_{15}\)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 dimethylsulfoxide (10 mL, 136 mmol, 900 mol %) in dry CH\(_2\)Cl\(_2\) (100 mL) at room temperature for 72 h. The mixture was then quenched with H\(_2\)O (120 mL) and diluted with CH\(_2\)Cl\(_2\) (350 mL). The organic phase was washed with brine (2 × 100 mL), dried over anhydrous Na\(_2\)SO\(_4\), 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 125–128 °C; \(R_f\) (50% EtOAc in hexane) 0.61; \(^1\)H NMR (CDCl\(_3\)) \(\delta\): 8.17 (dd, 1H, \(J = 7.8, 1.6, 1.0 \text{ Hz}\)), 8.04 (dd, 1H, \(J = 2.4, 1.6 \text{ Hz}\)), 7.94 (dd, 1H, \(J = 8.0, 1.3, 0.7 \text{ Hz}\)), 7.69 (dt, 1H, \(J = 8.2, 0.9 \text{ Hz}\)), 7.57–7.52 (m, 1H), 7.48–7.41 (m, 2H), 7.19 (dd, 1H, \(J = 8.1, 2.7, 0.9 \text{ Hz}\)), 5.74 (s, 1H); \(^13\)C NMR (CDCl\(_3\)) \(\delta\): 180.2, 157.1, 150.6, 140.6, 136.2, 130.0, 128.5, 128.3, 127.7, 123.2, 121.9, 117.9, 111.9; IR (KBr) 3463, 1651, 1593, 1525; Anal. (C\(_{16}\)H\(_{12}\)NO\(_2\)) C, H, N.

Benzo[d]oxazol-2-yl-(4-hydroxyphenyl)methanone (9b). Compound 6b (510 mg, 1.55 mmol, 100 mol %) and N-butylylpyridinium 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 bromide (820 mg, 3.8 mmol, 245 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 H\(_2\)O:25%aq ammonia (100 mL), H\(_2\)O (100 mL), and brine (100 mL). The organic phase was dried over anhydrous Na\(_2\)SO\(_4\), filtered, and evaporated to yield a yellow solid which was purified with flash chromatography (5% EtOAc in hexane) and recrystallization (EtOAc/hexane) giving 9b (310 mg, 86%) as a yellow solid: mp 188–189 °C; \(R_f\) (50% EtOAc in hexane) 0.60; \(^1\)H NMR (DMSO-\(d_6\)) \(\delta\): 10.80 (s, 1H), 8.45–8.42 (m, 2H), 8.04 (d, 1H, \(J = 7.9 \text{ Hz}\)), 7.93 (d, 1H, \(J = 8.2 \text{ Hz}\)), 7.66–7.62 (m, 1H), 7.57–7.53 (m, 1H), 7.02–6.99 (m, 2H); \(^13\)C NMR (DMSO-\(d_6\)) \(\delta\): 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. (C\(_{18}\)H\(_{14}\)NO\(_2\)) C, H, N.

**General Procedure for the Preparation of Phenolic Carbamates 11–26, 33, and 34 from 9a, 9b, and 10.** 3-Benzof[d]oxazol-2-carbonylphenyl ethylcarbamate (11). To a solution of benzof[d]oxazol-2-yl-(3-hydroxyphenyl)methanone (100 mg, 0.42 mmol, 100 mol %) in dry CH\(_2\)Cl\(_2\) (4 mL) were added triethylamine (60 \(\mu\)L, 0.42 mmol, 100 mol %) and ethyl isocyanate (166 \(\mu\)L, 2.1 mmol, 500 mol %). After stirring at room temperature for 12 h, the reaction was complete, as judged by TLC (5% EtO\(_2\) in CH\(_2\)Cl\(_2\)), and the mixture was diluted with EtOAc (8 mL), filtered through a pad of silica gel, and evaporated to dryness. Recrystal-
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; Rf (35% EtOAc in hexane) 0.49; [α]ᵢⁿ Tris-HCl, pH 7.4, with 1 mM EDTA and aliquoted for storage at −80 °C until use. The centrifuge was decapitated, 8 h after lights on (1500 h), whole brains were removed, dipped in isopentane on dry ice, and stored at −80 °C until use.

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 supernatant was used as a source of FAAH activity. The protein concentration of the final preparation, containing 5 μg membrane protein, 44 mM Tris-HCl (pH 7.4), 0.9 mM EDTA, 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, acetontitrile (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 acetontitrile) to stabilize compound 1 against acyl migration to (1S)-AG. Samples were centrifuged at 23 700 g for 4 min at room temperature prior to HPLC analysis of the supernatant.

In Vitro Assay for MGL Activity. The assay for MGL has been described previously.²⁶ 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 1. At time points of 0 and 90 min, 100 μL samples were removed from the incubation, acetontitrile (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 acetontitrile) to stabilize compound 1 against acyl migration to (1S)-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.²⁶ 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 acetontitrile was used at a flow rate of 2.0 mL min⁻¹. Retention times were 5.8 min for I, 6.3 min for (1S)-AG, and 10.2 min for arachidonic acid. The relative concentrations of I, (1S)-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.

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