# Modifications of the Ethanolamine Head in *N*-Palmitoylethanolamine: Synthesis and Evaluation of New Agents Interfering with the Metabolism of Anandamide

Séverine Vandevoorde,[†] Kent-Olov Jonsson,[‡] Christopher J. Fowler,[‡] and Didier M. Lambert*[,†]

*Unité de Chimie Pharmaceutique et de Radiopharmacie, Université Catholique de Louvain, Avenue Mounier, 73, UCL-CMFA 73.40, B-1200 Brussels, Belgium, and Department of Pharmacology and Clinical Neuroscience, Umeå University, SE-901 87 Umeå, Sweden*

*Received June 17, 2002*

The endogenous fatty acid amide anandamide (AEA) has, as a result of its actions on cannabinoid and vanilloid receptors, a number of important pharmacological properties including effects on nociception, memory processes, spasticity, and cell proliferation. Inhibition of the metabolism of AEA, catalyzed by fatty acid amide hydrolase (FAAH), potentiates the actions of AEA in vivo and therefore may be a useful target for drug development. In the present study, we have investigated whether substitution of the headgroup of the endogenous alternative FAAH substrate palmitoylethanolamide (PEA) can result in the identification of novel compounds preventing AEA metabolism. Thirty-seven derivatives of PEA were synthesized, with the C16 long chain of palmitic acid kept intact, and comprising 20 alkylated, 12 aromatic, and 4 halogenated amides. The ability of the PEA derivatives to inhibit FAAH-catalyzed hydrolysis of [$^3$H]AEA was investigated using rat brain homogenates as a source of FAAH. Inhibition curves were analyzed to determine the potency of the inhibitable fraction (p$I_{50}$ values) and the maximal attained inhibition for the compound, given that solubility in an aqueous environment is a major issue for these compounds. In the alkylamide family, palmitoylethylamide and palmitoylallylamide were inhibitors of AEA metabolism with p$I_{50}$ values of 5.45 and 5.47, respectively. Halogenated derivatives (Cl and Br) exhibit p$I_{50}$ values of ∼5.5 but rather low percentages of maximal inhibition. The −OH group of the ethyl head chain of *N*-palmitoylethanolamine was not necessary for interaction with FAAH. Amides containing aromatic moieties were less potent inhibitors of AEA metabolism. Compounds containing amide and ester bonds, **13** and **37**, showed p$I_{50}$ values of 4.99 and 5.08, respectively. None of the compounds showed obvious affinity for $CB_1$ or $CB_2$ receptors expressed on Chinese hamster ovary (CHO) cells. It is concluded that although none of the compounds were dramatically more potent than PEA itself at reducing the metabolism of AEA, the lack of effect of the compounds at $CB_1$ and $CB_2$ receptors makes them useful templates for development of possible therapeutic FAAH inhibitors.

## Introduction

Eleven years ago, a lipid derivative from arachidonic acid, *N*-arachidonoylethanolamine (AEA, Figure 1), was isolated from pig brain and was proposed as an endogenous ligand of cannabinoid receptors.[1] Considerable research has been devoted to this molecule, anandamide, and subsequent endogenous fatty acid amides or fatty acid esters congeners such as *N*-docosatetraenoylethanolamine, *N*-homo-γ-linolenoylethanolamine, and 2-arachidonoylglycerol (Figure 1) (for review see ref 2). AEA elicits a number of important pharmacological actions including effects on nociception,[3−7] memory processes,[8−9] lung function,[10] spasticity,[11] and cell proliferation[12,13] (for recent reviews of the therapeutic potential of AEA and related endocannabinoids, see refs 14 and 15). AEA not only acts as an endogenous ligand at cannabinoid receptors but is also capable of activating vanilloid receptors (VR).[16,17]

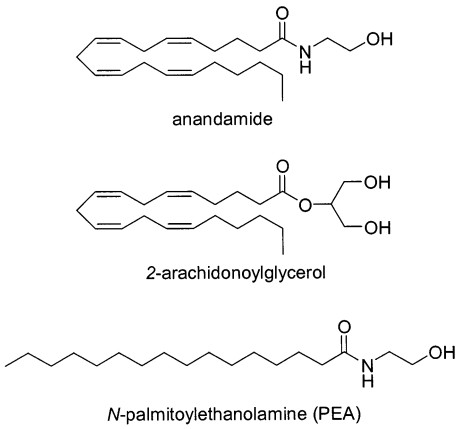



**Figure 1.** Structures of endocannabinoids and related endogenous lipids.

The pharmacological actions of AEA are terminated by its metabolism to arachidonic acid, catalyzed by fatty acid amide hydrolase (FAAH).[18] FAAH catalyzes the metabolism of not only AEA but other potentially important *N*-acylethanolamines and *N*-acylamides such

* To whom correspondence should be addressed. Phone: +32 2 764 7347. Fax: +32 2 764 73 63. E-mail: lambert@cmfa.ucl.ac.be.
[†] Université Catholique de Louvain.
[‡] Umeå University.

10.1021/jm0209679 CCC: $25.00 © 2003 American Chemical Society
Published on Web 03/19/2003

as anti-inflammatory agents palmitoylethanolamide, oleoylethanolamide (which may act as an anorexic lipid mediator regulated by feeding[19]), and the sleep-inducing agent oleamide;[20−22] for a review, see ref 23. Since FAAH inhibitors such as phenylmethylsulfonyl fluoride and palmitoylsulfonyl fluoride have been shown to potentiate the pharmacological actions of AEA both in vitro and in vivo,[24−26] FAAH is an interesting target for drug discovery. This conclusion has been strengthened by the recent finding that FAAH knockout mice show a large increase in brain AEA levels coupled with a reduced sensitivity to painful stimuli.[27] Very recently, the 2.8 Å crystal structure of the integral membrane protein FAAH has been reported,[28] which will facilitate the structure-based design of new inhibitors.

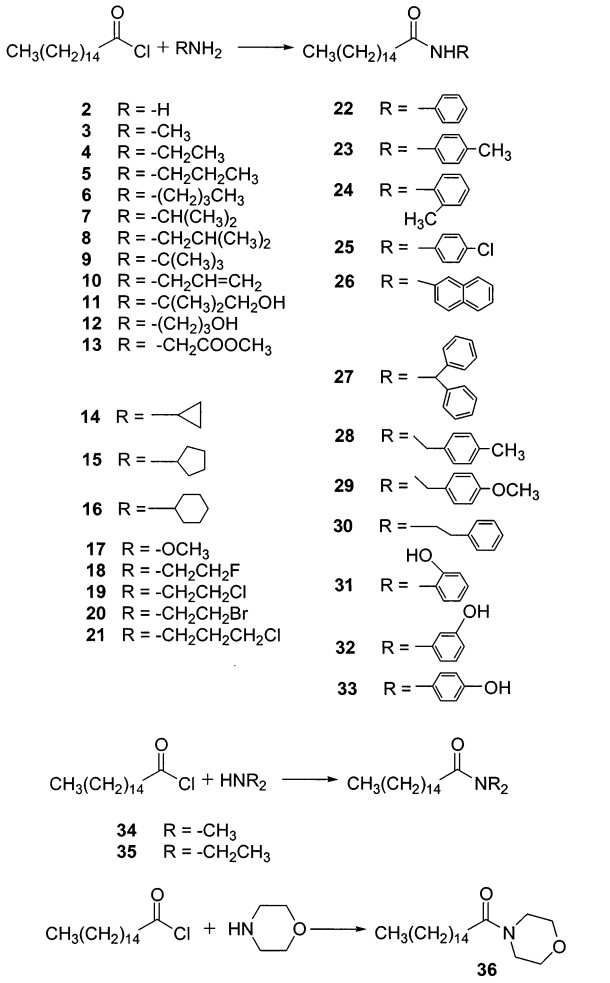
In 1985, Schmid et al.[22] reported that the chain length of the amino alcohol moiety of *N*-oleoylethanolamine dramatically affected its ability to interact with the amidohydrolase present in rat liver. This study clearly points to the ethyl head chain as a target for structure−activity studies. Indeed, considerable work has been done in this area, usually with arachidonoyl- and oleoylacyl chains, and resulted in the identification of several potent FAAH inhibitors (see, for example, refs 29−31), although an inherent disadvantage in this approach is that some of the compounds are per se active at cannabinoid receptors. In contrast, little is known about the effect of modification of the ethyl head chain in *N*-palmitoylethanolamine, despite the fact that this compound, which reduces the rate of metabolism of AEA by acting as a competing substrate,[32] is inactive at cannabinoid receptors.[33−35] Of the few palmitoyl compounds characterized, palmitoylsulfonyl fluoride (AM374) is a very potent FAAH inhibitor ($IC_{50} = 7$ nM) with little effect upon $CB_1$ cannabinoid receptors ($IC_{50} = 520$ nM),[36] and this compound has proven to be useful in vivo.[11] There is thus a need to further investigate the effect of substitution of the ethyl head chain of *N*-palmitoylethanolamine upon its ability to interact with FAAH. To this end, we have initiated a project investigating the biochemistry of analogues and homologues of *N*-palmitoylethanolamine. In our initial studies, we found that the homologues, which were devoid of affinity for either $CB_1$ or $CB_2$ receptors, were able (1) to prevent the FAAH-catalyzed metabolism of AEA[37] and (2) to potentiate anandamide activation by VR1 receptors expressed on hVR1-HEK293 cells.[38,39] Such effects, whereby inactive endogenous compounds potentiate the effects of related endogenous active compounds, were first reported for the 2-lineoyl- and 2-palmitoyl homologues of 2-arachidonoylglycerol and termed an "entourage" effect.[40]

In contrast to our knowledge concerning the homologues, only six analogues of *N*-palmitoylethanolamine (compounds **2**, **4**, **6**, **7**, and **16** shown here and *R*-palmitoyl(2-methyl)ethanolamide) were characterized in these studies. In consequence, in the present study, we have investigated in detail the ability of a series of *N*-palmitoylethanolamine analogues to interact with FAAH and $CB_1$ and $CB_2$ receptors.

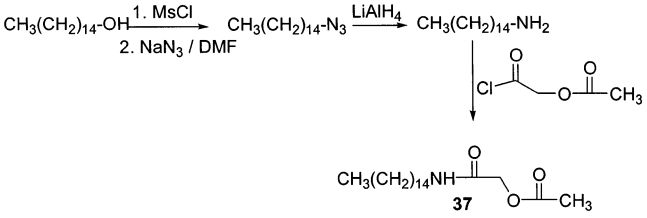
## Results and Discussion

**Chemistry.** Palmitoylethanolamide **1** analogues **2**−**36**, varying by a modification of the hydroxylamino moiety,

**Scheme 1.** Syntheses of Primary (**2**), Secondary (**3**−**33**), and Tertiary (**34**−**36**) Amides




**Scheme 2.** Synthesis of *N*-(2-Acetoxyacetyl)pentadecylamine **37**




have been prepared in good yields following the scheme from palmitoyl chloride and the respective amines (Scheme 1). They include primary amides (**2**), secondary amides (**3**−**33**), and halogen and tertiary amides (**34**−**36**). *N*-(2-Acetoxyacetyl)pentadecylamine (**37**) was prepared from pentadecylamine and acetoxyacetyl chloride (Scheme 2).

**Pharmacology.** Compounds **1**−**37** have been evaluated in an FAAH assay using rat brain homogenates as the source of the enzyme and [$^3$H]-anandamide as the labeled substrate. The amount of [$^3$H]-ethanolamine released has been determined after chloroform/methanol extraction. It is important to stress at the outset that the observed absolute inhibition of AEA metabolism found with a given concentration of the compounds reflects not only their potency but also their solubility in the assay medium. The latter is an important issue,

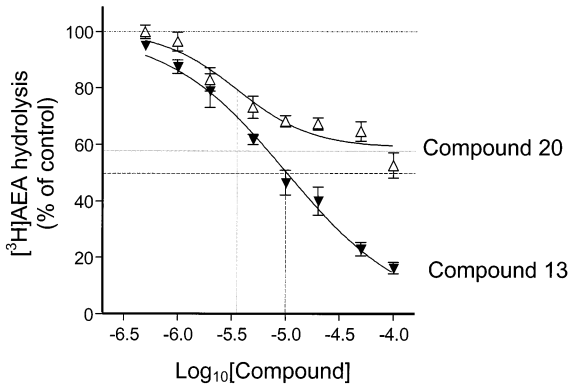



**Figure 2.** Inhibition of 2 $\mu$M [$^3$H]-AEA metabolism by compounds **13** (filled triangles) and **20** (open triangles). For compound **13**, analysis of the data indicated that the maximum inhibition was not significantly different from 100% (i.e., 95% confidence limits of the minimum activity remaining straddled 0%). The p$I_{50}$ value of the inhibitable fraction is thus the concentration of the compound that produces 50% inhibition of [$^3$H]-AEA metabolism (shown as broken line in the figure). For compound **20**, the analysis indicated that the curve reached a plateau at a value significantly higher than zero (59%, 95% confidence limits 52−66%). Thus, the inhibitable fraction of the activity is 41%. The p$I_{50}$ value of the inhibitable fraction is thus the concentration of the compound that produces 20.5% inhibition of [$^3$H]-AEA metabolism (shown as ⋯ in the figure). The data are the mean ± SEM, $n = 3$.

particularly for saturated long-chain *N*-acylethanolamides and their analogues. To separate potency from solubility, the inhibition curves were analyzed using a fixed maximum value (i.e., 100% of control) and a floating bottom value (reflecting the maximum attainable inhibition). The p$I_{50}$ value of the inhibitable component was then determined. An example of this is shown for compounds **13** and **20** in Figure 2. In the discussion below, we have mainly focused upon the p$I_{50}$ values being the measure of the potency of the compounds, although of course the goal is to find compounds that are potent and are without solubility problems.

The results are presented in Tables 1 and 2. *N*-Palmitoylethanolamine inhibited FAAH with a p$I_{50}$ value of 5.30 (corresponding to an $IC_{50}$ value of 5.1 $\mu$M), in good agreement with the $K_M$ value of 2.9 $\mu$M found in the rat brain using the same assay for this compound as a substrate of FAAH.[41] However, the $K_M$ values are highly dependent on the assay conditions used (variations in the $K_M$ values for AEA between 0.8 and 180 $\mu$M have been reported in the literature[23]), and thus, it is relevant only to compare potencies when the same assay methodology is used.

Replacement of the −OH group in the ethyl head chain by a halogen atom in the ethyl head chain (**18**−**21**) had little effect on the potency, with p$I_{50}$ values being in the 5.45−5.60 range. Replacement of the −OH group by a hydrogen results in compound **4**, which exhibits a potency similar to that observed with **1**. A similar relation was seen with the oleoylacyl group homologues (present authors, unpublished data). This indicates that the hydroxyl group, albeit a donor−acceptor group for the H bond, is not crucial for the FAAH inhibition. This conclusion can also be drawn for chains with three carbon atoms in the headgroup, since compounds **6** and **12** show similar p$I_{50}$ values. These compounds illustrate the importance of separating p$I_{50}$ values from maximum inibition in the analysis. If the inhibition curves had been interpreted per se, a different conclusion would have been made given the considerable difference in the attainable maximum inhibition of compounds **6** and **12** (Table 1).

The results shown above are entirely consistent with the situation for arachidonoyl-based compounds. Thus, Jarrahian et al.[42] reported that replacement of the ethanolamine headgroup of AEA with a cyclopropyl, methylcyclopropyl, or cyclobutyl group resulted in active compounds capable of inhibiting the metabolism of [$^{14}$C]-AEA by rat forebrain FAAH. Similarly, the chloroethyl and chloromethyl analogues were active in this respect.[42] Interestingly, Lang et al.[43] reported that while the fluoroethyl analogue of AEA was metabolized by FAAH (and therefore inhibits AEA metabolism by acting as a competing substrate), the isopropyl and *tert*-butyl analogues were not metabolized. Thus, requirements for interaction with and metabolism by FAAH are rather different, and compounds such as **7** are presumably acting as true inhibitors rather than as alternative substrates. The lack of an absolute requirement of the −OH group for interaction with FAAH is reminiscent of the situation for the interaction of arachidonoyl analogues with $CB_1$ receptors[44,45] and may thus be a general phenomenon for the endocannabinoid system.

Elongation of compound **4** with one (compound **5**) and two methylene groups (**6**) did not affect the p$I_{50}$ values, whereas a decrease of the number of carbon atoms (**3**) reduced the p$I_{50}$ value. The primary amide **2**, however, had a p$I_{50}$ value of 5.01. Compound **10**, featuring a double bond in the headgroup, exhibited a potency similar to that of *N*-palmitoylethanolamine. The increase of steric hindrance by the introduction of two methyl groups at the α-carbon on the amide group yielded an inactive compound (**11**), but this result, when taken together with the results obtained for the cycloalkyl substituents (**14**−**16**) and to some extent with some aryl groups (**22**, **24**, **26**), indicates that the absence of inhibition is not simply due to a bulky steric effect.

The case of the aryl substituents merits further attention. Indeed, the steric parameters seem to be very tight. Para substitution on the aniline is unfavorable (**23**, **25**), whereas a short spacer between the aromatic group and the palmitic side chain resulted in a better inhibitor (**28**). Given that many phenolic arachidonic acid derivatives (arachidonoylserotonin,[29] AM-404,[37] and *N*-arachidonoyldopamine[46]) are mixed inhibitors of FAAH, the ortho (**31**), meta (**32**), and para phenol (**33**) derivatives have been prepared in order to obtain a stronger inhibitory potential. However, these three compounds resulted in weaker inhibitors compared to compound **22**. In addition, the position of the hydroxyl group does not seem to be as crucial as for the case of AM404. Modifications of the amide bond by steric hindrance (the dimethyl compound **11**) or by suppression of the hydrogen on the NH (tertiary amides **34**−**36**) lead to a drastic decrease of the FAAH inhibition potential. The latter finding underlines the importance of the amide bond because it occurs in the endogenous compounds.

In a separate series of experiments, compounds **1**, **7**, **12**, and **13** were assessed for their ability to inhibit AEA

**Table 1.** Inhibition of AEA Metabolism and Interaction with CB Receptors for Analogues of Palmitoylethanolamide (**1**)[a]

| | inhibition of AEA metabolism | | $CB_1$ (% inhibition of specific binding of [$^3$H]-CP55,940), [compound] = 10 $\mu$M | $CB_2$ (% inhibition of specific binding of [$^3$H]-Win55,212-2), [compound] = 10 $\mu$M |
|---|---|---|---|---|
| compd[b] | p$I_{50}$ | max of inhibition obtained, % | | |
| **1*** | 5.30 ± 0.15 | 78 ± 7 | 23.8 ± 0.07 | 13.9 ± 1.7 |
| **2** | 5.01 ± 0.18 | 51 ± 7 | 11.7 ± 0.3 | 12.9 ± 0.4 |
| **3** | 4.57 ± 0.06 | 100 | 2.5 ± 0.5 | 7.8 ± 0.1 |
| **4**# | 5.45 ± 0.04 | 72 ± 2 | 2.8 ± 0.3 | 10 ± 0.4 |
| **5** | 5.56 ± 0.05 | 49 ± 2 | 14.4 ± 0.6 | 6.1 ± 0.2 |
| **6*** | 5.54 ± 0.16 | 24 ± 3 | 19.6 ± 3.4 | 12.9 ± 0.4 |
| **7*** | 4.89 ± 0.27 | 61 ± 11 | 25.4 ± 4.5 | 12.3 ± 0.8 |
| **8** | | 6% inhibition @ 100 $\mu$M | 18.5 ± 0.4 | 8.9 ± 0.2 |
| **9** | | 0% inhibition @ 100 $\mu$M | 24.6 ± 0.5 | 2.0 ± 0.3 |
| **10** | 5.47 ± 0.06 | 67 ± 3% | 13.3 ± 0.4 | 7.8 ± 0.3 |
| **11** | | 0% inhibition @ 100 $\mu$M | 15.7 ± 0.3 | 6.7 ± 0.2 |
| **12** | 5.42 ± 0.07 | 61 ± 3 | 15.0 ± 1.1 | 6.4 ± 0.1 |
| **13** | 4.99 ± 0.04 | 100 | 7.3 ± 0.5 | 4.1 ± 0.2 |
| **14** | 5.35 ± 0.10 | 57 ± 4 | 12.8 ± 0.3 | 16.3 ± 0.5 |
| **15** | ~3.8 | 43% inhibition @ 100 $\mu$M | 27.1 ± 0.7 | 9.5 ± 0.1 |
| **16*** | | 11% inhibition @ 100 $\mu$M | 34.2 ± 1.5 | 8.1 ± 0.2 |
| **17** | ~3.9 | 46% inhibition @ 100 $\mu$M | 18.1 ± 0.6 | 5.2 ± 0.3 |
| **18** | | 6% inhibition @ 100 $\mu$M | 8.2 ± 0.3 | 3.9 ± 0.3 |
| **19** | 5.58 ± 0.09 | 48 ± 3 | 25.2 ± 1.0 | 13.5 ± 0.5 |
| **20** | 5.45 ± 0.11 | 41 ± 3 | 35.8 ± 1.1 | 15.1 ± 0.7 |
| **21** | 5.60 ± 0.07 | 27 ± 1 | 30.6 ± 0.4 | 14.3 ± 0.6 |
| **22** | 5.31 ± 0.11 | 18 ± 2 | 2.8 ± 0.2 | 0.5 ± 0.2 |
| **23** | | 0% inhibition @ 100 $\mu$M | 2.8 ± 0.2 | 6.5 ± 0.1 |
| **24** | 4.85 ± 0.18 | 31 ± 6 | 4.9 ± 0.3 | 6.5 ± 0.2 |
| **25** | | 11% inhibition @ 100 $\mu$M | 11.1 ± 0.5 | 7.4 ± 0.3 |
| **26** | 4.70 ± 0.24 | 28 ± 8 | 0.7 ± 0.1 | 0.1 ± 0.0 |
| **27** | | 0% inhibition @ 100 $\mu$M | 2.2 ± 0.1 | 1.1 ± 0.2 |
| **28** | 4.07 ± 0.07 | 100 | 4.8 ± 0.4 | 0.7 ± 0.1 |
| **29** | ~3.7 | 29% inhibition @ 100 $\mu$M | 25.4 ± 0.6 | 3.7 ± 0.5 |
| **30** | | 19% inhibition @ 100 $\mu$M | 22.8 ± 0.9 | 1.3 ± 0.2 |
| **31** | 4.40 ± 0.01 | 53 ± 5 | 10.8 ± 0.2 | 2.7 ± 0.1 |
| **32** | 4.27 ± 0.03 | 51 ± 2 | 40.9 ± 1.2 | 3.9 ± 0.7 |
| **33** | 4.15 ± 0.06 | 55 ± 3 | 44.8 ± 1.7 | 5.0 ± 0.6 |
| **34** | ~3.6 | 30% inhibition @ 100 $\mu$M | 0.3 ± 0.1 | 6.4 ± 0.4 |
| **35** | | 2% inhibition @ 100 $\mu$M | 3.3 ± 0.3 | 2.7 ± 0.1 |
| **36** | | 6% inhibition @ 100 $\mu$M | 15.5 ± 0.3 | 4.2 ± 0.3 |
| **37** | 5.08 ± 0.05 | 87 ± 4 | 16.0 ± 0.8 | 6.0 ± 0.3 |

[a] Values are calculated from a minimum of three experiments. [b] The asterisk (*) indicates data from Jonsson,[37] and the pound sign (#) indicates data from Smart [FAAH only][38].

**Table 2.** Inhibition of AEA Metabolism by Compounds **1**, **7**, **12**, and **13** at Different Assay pH[a]

| | p$I_{50}$ value | | maximum attainable inhibition, % | |
|---|---|---|---|---|
| compd | pH 6 | pH 9 | pH 6 | pH 9 |
| **1** | 4.93 ± 0.09 | 5.04 ± 0.06 | 62 ± 6 | 100 |
| **7** | | 4.85 ± 0.17 | 4 ± 9% at 50 $\mu$M | 39 ± 10 |
| **12** | 5.01 ± 0.07 | 5.16 ± 0.10 | 45 ± 5 | 81 ± 8 |
| **13** | 4.86 ± 0.14 | 4.72 ± 0.09 | 75 ± 11 | 100 |

[a] p$I_{50}$ values and maximal attainable inhibitions were calculated from three to five experiments (using three homogenate preparations) over a concentration range of 3−100 $\mu$M (seven concentrations). For **7**, the highest concentration gave precipitation, so it was not used in the analysis.

metabolism at two assay pH values, pH 6 and pH 9 (Table 2). This approach was undertaken in view of the finding that potency of inhibition of AEA metabolism can show pH dependency.[47] In the case of compounds **1**, **12**, and **13**, the p$I_{50}$ values were similar at the two pH values, and the values were in reasonable agreement with those found at pH 7.6 (Table 1) given the fact that the time elapsed between the two sets of experiments was greater than 9 months. In contrast, the maximum observable inhibition was greater at pH 9 than at pH 6, again underlining the importance of separating these two parameters in the analyses. Compound **7** showed little effect at pH 6. Thus, the solubilities of the compounds show pH dependency, but not their potencies.

The affinity for cannabinoid receptors was determined for the entire set of compounds in radioligand binding assays using homogenates of Chinese hamster ovary (CHO) cell membranes selectively expressing either the human $CB_1$ or the human $CB_2$ cannabinoid receptors. The results of the screening of compounds **1**−**37** at 10 $\mu$M are given in Table 1 and are expressed as percentages of inhibition of the specific binding of the radioligand used. All the compounds were essentially devoid of $CB_2$ affinity. None of the palmitoyl compounds with the exception of compounds **16**, **20, 21, 32**, and **33** exhibit an inhibition of specific binding greater than 30% at the $CB_1$ receptor. Even in these three compounds, the inhibition did not exceed 45%.

From the present study, it can be concluded that the −OH group of the ethyl head chain of *N*-palmitoylethanolamine is not necessary for interaction with FAAH. However, there are considerable steric restrictions placed upon the ability of analogues of *N*-palmitoylethanolamine to interact with the enzyme. Although these data shed considerable light upon the structure−activity relationships for the ethyl headgroup and the palmitoyl acyl side chain, the substitutions have led either to an unchanged or to a loss of affinity for FAAH rather than to a dramatic gain. Thus, the most potent compound of the study, **21**, with a p$I_{50}$ value of 5.60, is considerably less potent an inhibitor of FAAH than either its sulfonyl fluoride (AM274, p$I_{50}$ = 8.15 [36]) or

trifluoromethyl ketone (PTMK, p$I_{50}$ = 7.10 [37]) analogues (see refs 36 and 48 for structure−activity data on these side groups with variations in saturated acyl side chain length). Nevertheless, the lack of effect of the compounds at $CB_1$ and $CB_2$ receptors makes them useful templates for development of possible therapeutic FAAH inhibitors.

## Experimental Section

**Chemistry.** All solvents and reagents were obtained from commercial suppliers and used without further purification unless otherwise noted. Analytical thin-layer chromatography (TLC) was undertaken using Merck silica gel $F_{254}$ plates with detection under UV lamp or iodine vapor. Flash chromatography was performed using silica gel (60 Å, 40−63 $\mu$m, 230−400 mesh ASTM) from ROCC. Thin-layer chromatography was used to designate the tubes containing the purified product.

Melting points (mp) were determined in open capillaries using an Electrothermal 9100 apparatus and are reported uncorrected. Infrared (IR) spectra were recorded using a Perkin-Elmer FT-IR 286 spectrometer, and values are reported as frequency ($\nu$) and expressed in $cm^{-1}$. Nuclear magnetic resonance ($^1H$ NMR, $^{13}C$ NMR) spectra were recorded on a Bruker AM-300 spectrometer and analyzed using the WIN-NMR software package. Chemical shifts ($\delta$) are reported relative to the tetramethylsilane peak set up at 0.00 ppm. They were quoted in the case of multiplets by their interval values. Signals were designed as the following: s, singlet; d, doublet; t, triplet; m, multiplet. Coupling constants were expressed in hertz. EI mass spectra were obtained on a Finnigan MAT 44S, with an ionization voltage of 70 eV. CAS numbers are indicated below when known. Elemental analyses were performed on a Carlo Erba EA 1108 analyzer (Carlo-Erba, Milano, Italy) and are within ±0.4% of the theoretical values.

**Alkylated Amides. Synthesis of Hexadecanamide (2).** This compound has been prepared as previously described:[49] mp 104−104.4 °C (uncorrected); TLC (chloroform/methanol 8:2 $vv^{-1}$) $R_f$ = 0.8; $^1H$ NMR ($CDCl_3$) $\delta$ (ppm) 0.88 (t, $J$ = 6 Hz, 3H), 1.21−1.42 (m, 26 H), 2.22 (t, $J$ = 7 Hz, 2H), 5.42 (NH); $^{13}C$ NMR ($CDCl_3$) $\delta$ (ppm) 14.12 ($CH_3$), 22.7, 25.57, 29.27, 29.37, 29.50, 29.63, 29.71, 31.94, 35.98 ($CH_2$), 175.59 (C═O); mass spectrometry [$M^{+\bullet}$] = 256; IR $\nu$ ($cm^{-1}$) 3353 (NH), 1632 (C═O). These values were consistent with those reported in the literature.[50] CAS number: 629-54-9.

**Synthesis of *N*-Methylhexadecanamide (3).** In a two-neck flask, 5 mL of methylamine (40% solution in water) was poured into 10 mL of dry methylene chloride. The solution was cooled in an ice bath and magnetically stirred. Palmitoyl chloride (2.74 g, 10 mmol) was added dropwise. The reaction mixture was stirred for 12 h at room temperature and then washed with 5% sodium bicarbonate solution, 1 M HCl, and brine. The organic layer was dried over $MgSO_4$, and after filtration, the solvent was evaporated under reduced pressure to give 2.67 g (99%) of a white solid: mp 98.6−99.8 °C (uncorrected); TLC (chloroform/methanol 8:2 $vv^{-1}$) $R_f$ = 0.88; $^1H$ NMR ($CDCl_3$) $\delta$ (ppm) 0.88 (t, $J$ = 3 Hz, 3H), 1.16−1.34 (m, 26 H), 2.1 (t, $J$ = 7 Hz, 2H), 2.6−2.9 (m, 3H), 5.44 (NH); $^{13}C$ NMR ($CDCl_3$) $\delta$ (ppm) 14.1 ($CH_3$), 22.7, 25.8, 26.26, 29.37, 29.5, 29.63, 29.66, 29.69, 31.94 ($CH_2$), 36.78 ($CH_3$), 173.78 (C═O); mass spectrometry [$M^{+\bullet}$] = 270; IR $\nu$ ($cm^{-1}$) 3299 (NH), 1635 (C═O). CAS number: 7388-58-1.

**Synthesis of *N*-Propylhexadecanamide (5).** The procedure described for compound **3** was used with 5.9 g (100 mmol) of propylamine and 2.74 g (10 mmol) of palmitoyl chloride to give 2 g (68%) of a white solid: mp 65.4−66 °C (uncorrected); TLC (chloroform/methanol 8:2 $vv^{-1}$) $R_f$ = 0.87; $^1H$ NMR ($CDCl_3$) $\delta$ (ppm) 0.87 (t, $J$ = 3 Hz, 3H), 1.18−1.35 (m, 28 H), 1.58−1.62 (m, 3H), 2.1 (t, $J$ = 7 Hz, 2H), 3.18−3.24 (m, 2H), 5.49 (NH); $^{13}C$ NMR ($CDCl_3$) $\delta$ (ppm) 11.37, 14.1 ($CH_3$), 22.7, 22.98, 25.89, 29.37, 29.4, 29.55, 29.69, 29.72, 31.97, 36.99, 41.21 ($CH_2$), 173.1 (C═O); mass spectrometry [$M^{+\bullet}$] = 298; IR $\nu$ ($cm^{-1}$) 3296 (NH), 1637 (C═O). CAS number: 189939-61-5.

**Synthesis of *N*-(2-Methylpropyl)hexadecanamide (8).** The procedure described for compound **3** was used with 4.8 g (66 mmol) of isobutylamine and 1.8 g (6.6 mmol) of palmitoyl chloride to give 1.95 g (95%) of a white solid: mp 68−69.6 °C (uncorrected); TLC (chloroform/methanol 8:2 $vv^{-1}$) $R_f$ = 0.82; $^1H$ NMR ($CDCl_3$) $\delta$ (ppm) 0.89 (t, $J$ = 6 Hz, 9H), 1.10−1.30 (m, 26 H), 1.48−1.52 (m, 2H), 2.1−2.19 (m, 1H), 3.05−3.09 (m, 2H), 5.56 (NH); $^{13}C$ NMR ($CDCl_3$) $\delta$ (ppm) 14.1 ($CH_3$), 20.10, 22.71, 25.92, 28.54, 29.35, 29.52, 29.63, 29.66, 29.69, 31.95, 37.00 ($CH_2$), 46.82 (CH), 173.15 (C═O); mass spectrometry [$M^{+\bullet}$] = 312; IR $\nu$ ($cm^{-1}$) 3315 (NH), 1641 (C═O). CAS number: 54794-72-8.

**Synthesis of *N*-(1,1-Dimethylethyl)hexadecanamide (9).** The procedure described for compound **3** was used with 7.3 g (100 mmol) of *tert*-butylamine and 2.74 g (10 mmol) of palmitoyl chloride to give 1.05 g (34%) of a white solid: mp 52−54 °C (uncorrected); TLC (chloroform/methanol 6:4 $vv^{-1}$) $R_f$ = 0.9; $^1H$ NMR ($CDCl_3$) $\delta$ (ppm) 0.87 (t, $J$ = 6 Hz, 12H), 1.10−1.45 (m, 26 H), 2.07 (t, $J$ = 7 Hz, 2H), 5.34 (NH); $^{13}C$ NMR ($CDCl_3$) $\delta$ (ppm) 14.12($CH_3$), 22.71, 25.84, 28.88, 29.26, 29.38, 29.42, 29.53, 29.64, 29.68, 29.71, 31.94, 37.78 ($CH_2$), 51.01 (C), 172.55 (C═O); mass spectrometry [$M^{+\bullet}$] = 312; IR $\nu$ ($cm^{-1}$) 3325 (NH), 1643 (C═O). CAS number: 74058-70-1.

**Synthesis of *N*-(2-Propenyl)hexadecanamide (10).** The procedure described for compound **3** was used with 5.7 g (100 mmol) of allylamine and 2.74 g (10 mmol) of palmitoyl chloride to give 1.74 g (59%) of a white solid: mp 61−63 °C (uncorrected); TLC (ethyl acetate/methanol 8:2 $vv^{-1}$) $R_f$ = 0.77; $^1H$ NMR ($CDCl_3$) $\delta$ (ppm) 0.87 (t, $J$ = 3 Hz, 3H), 1.22−1.54 (m, 26 H), 2.19 (t, $J$ = 7 Hz, 2H), 3.7−3.72 (m, 2H), 5.11−5.2 (m, 2H), 5.55 (NH), 5.79−5.88 (m, 1H); $^{13}C$ NMR ($CDCl_3$) $\delta$ (ppm) 14.10 ($CH_3$), 22.77, 25.88, 29.43, 29.56, 29.76, 32.02, 35.39, 36.87, 41.99, 58.48, 116.39 ($CH_2$), 134.51 (CH), 173.07 (C═O); mass spectrometry [$M^{+\bullet}$] = 296; IR $\nu$ ($cm^{-1}$) 3299 (NH), 1636 (C═O). CAS number: 1012114-99-8.

**Synthesis of *N*-(2-Hydroxy-1,1-dimethylethyl)hexadecanamide (11).** The procedure described for compound **3** was used with 8.9 g (100 mmol) of 2-amino-2-methylpropan-1-ol and 2.74 g (10 mmol) of palmitoyl chloride to give 1.11 g (34%) of a white solid: mp 65−68 °C (uncorrected); TLC (ethyl acetate/hexane 6:4 $vv^{-1}$) $R_f$ = 0.51; $^1H$ NMR ($CDCl_3$) $\delta$ (ppm) 0.89 (t, $J$ = 6 Hz, 9H), 1.22−1.42 (m, 26 H), 2.14 (t, $J$ = 7 Hz, 2H), 3.55 (s, 2H), 4.86 (NH); $^{13}C$ NMR ($CDCl_3$) $\delta$ (ppm) 14.42 ($CH_3$), 23.68, 24.19, 27.04, 30.21, 30.41, 30.73, 32.99, 37.72, 69.55 ($CH_2$), 56.03 (C), 176.37 (C═O); mass spectrometry [$M^{+\bullet}$] = 328; IR $\nu$ ($cm^{-1}$) 3425 (OH), 3295 (NH), 1625 (C═O). CAS number: 36136-10-4.

**Synthesis of *N*-Palmitoyl-3-amino-1-propanol (12).** The procedure described for compound **3** was used with 7.5 g (100 mmol) of 2-amino-2-methylpropan-1-ol and 2.74 g (10 mmol) of palmitoyl chloride to give 1.67 g (54%) of a white solid: mp 83−84 °C (uncorrected); TLC (acetone/methylene chloride 1:1 $vv^{-1}$) $R_f$ = 0.55; $^1H$ NMR ($CDCl_3$) $\delta$ (ppm) 0.88 (t, $J$ = 7 Hz, 3H), 1.11−1.34 (m, 26 H), 1.60−1.67 (m, 4H), 2.19 (t, $J$ = 6 Hz, 2H), 3.41−3.43 (m, 2H), 5.8 (NH); $^{13}C$ NMR ($CDCl_3$) $\delta$ (ppm) 14.10 ($CH_3$), 22.71, 25.81, 29.31, 29.50, 29.69, 32.48, 36.23, 36.75, 59.32 ($CH_2$), 174.49 (C═O); mass spectrometry [$M^{+\bullet}$] = 312; IR $\nu$ ($cm^{-1}$) 3428 (OH), 3297 (NH), 1636 (C═O). CAS number: 18704-66-0.

**Synthesis of *N*-(1-Oxohexadecyl)glycine Methyl Ester (13).** The procedure described for compound **3** was used with 6.27 g (50 mmol) of glycine methyl ester hydrochloride, 8.2 mL (60 mmol) of triethylamine, and 2.74 g (10 mmol) of palmitoyl chloride to give 3.23 g (99%) of a white solid: mp 158−162 °C (uncorrected); TLC (ethyl acetate/methanol 8:2 $vv^{-1}$) $R_f$ = 0.75; $^1H$ NMR ($CDCl_3$) $\delta$ (ppm) 0.88 (t, $J$ = 7 Hz, 3H), 1.12−1.35 (m, 26 H), 2.24 (t, $J$ = 7 Hz, 2H), 3.2 (s, 3H), 3.71−3.83 (m, 2H), 4.8 (NH); $^{13}C$ NMR ($CDCl_3$) $\delta$ (ppm) 14.42 ($CH_3$), 23.74, 26.85, 30.28, 30.47, 30.79, 33.12, 36.81, 41.01, 41.86 ($CH_2$), 49.62 ($CH_3$), 168.99, 178.00 (C═O); mass spectrometry [$M^{+\bullet}$] = 328; IR $\nu$ ($cm^{-1}$) 3310 (NH), 1641 (C═O), 1747 (C═O). CAS number: 214706-34-0.

**Synthesis of *N*-Cyclopropylhexadecanamide (14).** The procedure described for compound **3** was used with 5.7 g (100

mmol) of cyclopropylamine and 2.74 g (10 mmol) of palmitoyl chloride to give 2.16 g (73%) of a white solid: mp 87−89 °C (uncorrected); TLC (chloroform/acetone 9:1 vv$^{-1}$) $R_f$ = 0.48; $^1$H NMR ($CDCl_3$) $\delta$ (ppm) 0.88 (t, $J$ = 7 Hz, 3H), 1.11−1.43 (m, 26 H), 1.60−1.71 (m, 4H), 2.11 (t, $J$ = 7 Hz, 2H), 3.71−3.8 (m, 1H), 5.59 (NH); $^{13}$C NMR ($CDCl_3$) $\delta$ (ppm) 14.10 ($CH_3$), 22.58, 22.71, 25.75, 29.37, 29.50, 29.69 ($CH_2$), 31.96 (CH), 36.68 ($CH_2$), 174.49 (C═O); mass spectrometry [M$^{+\bullet}$] = 296; IR $\nu$ (cm$^{-1}$) 3295 (NH), 1640 (C═O). Anal. ($C_{19}H_{37}NO$) C, H, N.

**Synthesis of *N*-Cyclopentylhexadecanamide (15).** The procedure described for compound **3** was used with 8.5 g (100 mmol) of cyclopentylamine and 2.74 g (10 mmol) of palmitoyl chloride to give 2.36 g (73%) of a white solid: mp 64−65 °C (uncorrected); TLC (ethyl acetate/hexane 1:1 vv$^{-1}$) $R_f$ = 0.61; $^1$H NMR ($CDCl_3$) $\delta$ (ppm) 0.88 (t, $J$ = 7 Hz, 3H), 1.11−1.42 (m, 26 H), 2.01−2.09 (m, 4H), 2.14 (t, $J$ = 7 Hz, 2H), 2.25−2.28 (m, 4H), 4.0−4.24 (m, 1H), 5.34 (NH); $^{13}$C NMR ($CDCl_3$) $\delta$ (ppm) 14.16 ($CH_3$), 22.77, 23.81, 24.91, 25.03, 25.94, 29.44, 29.56, 29.76, 31.96, 33.25, 33.83, 34.48, 37.07 ($CH_2$), 51.17 (CH), 172.74(C═O); mass spectrometry [M$^{+\bullet}$] = 324; IR $\nu$ (cm$^{-1}$) 3303 (NH), 1639 (C═O). Anal. ($C_{21}H_{41}NO$) C, H, N.

**Synthesis of *N*-Methoxyhexadecanamide (17).** The procedure described for compound **3** was used with 4.2 g (50 mmol) of methoxylamine hydrochloride, 8.2 mL (60 mmol) of triethylamine, and 2.74 g (10 mmol) of palmitoyl chloride to give 2.28 g (80%) of a white solid: mp 52−53 °C (uncorrected); TLC (chloroform/methanol 6:4 vv$^{-1}$) $R_f$ = 0.88; $^1$H NMR ($CDCl_3$) $\delta$ (ppm) 0.88 (t, $J$ = 7 Hz, 3H), 1.16−1.52 (m, 26 H), 2.43 (t, $J$ = 7 Hz, 2H), 3.47−3.49 (m, 3H); $^{13}$C NMR ($CDCl_3$) $\delta$ (ppm) 14.12 ($CH_3$), 22.74, 24.31, 25.02, 28.93, 29.24, 29.41, 29.45, 29.63, 29.75, 31.98, 34.17, 35.35 ($CH_2$), 51.40 ($CH_3$), 169.63(C═O); mass spectrometry [M$^{+\bullet}$] = labile; IR $\nu$ (cm$^{-1}$) 1640 (C═O). CAS number: 337962-52-4.

**Halogenated Amides. Synthesis of *N*-(2-Fluoroethyl)-hexadecanamide (18).** The procedure described for compound **3** was used with 300 mg (3 mmol) of 2-fluoroethylamine hydrochloride, 500 $\mu$L of triethylamine (3.65 mmol), and 82 mg (0.3 mmol) of palmitoyl chloride to give 70 mg (78%) of a white solid: mp 54.6−55.9 °C (uncorrected); TLC (chloroform/acetone 9:1 vv$^{-1}$) $R_f$ = 0.5; $^1$H NMR ($CDCl_3$) $\delta$ (ppm) 0.86 (t, $J$ = 6 Hz, 3H), 1.15−1.43 (m, 26 H), 2.31 (t, $J$ = 7 Hz, 2H), 2.41−2.43 (m, 2H), 4.37−4.39 (m, 2H); $^{13}$C NMR ($CDCl_3$) $\delta$ (ppm) 14.10 ($CH_3$), 22.71, 24.26, 24.72, 28.92, 29.18, 29.37, 29.69, 31.96, 33.71, 35.33, 73.95 ($CH_2$), 169.64 (C═O); mass spectrometry [M$^{+\bullet}$] =302; IR $\nu$ (cm$^{-1}$) 3306 (NH), 1643(C═O). Anal. ($C_{18}H_{36}FNO$) C, H, N.

**Synthesis of *N*-(2-Chloroethyl)hexadecanamide (19).** The procedure described for compound **3** was used with 1.16 g (10 mmol) of 2-chloroethylamine hydrochloride, 3 mL of triethylamine (22 mmol), and 274 mg (1 mmol) of palmitoyl chloride to give 254 mg (80%) of a white solid: mp 82.2−82.5 °C (uncorrected); TLC (ethyl acetate/methanol 8:2 vv$^{-1}$) $R_f$ = 0.9; $^1$H NMR ($CDCl_3$) $\delta$ (ppm) 0.87 (t, $J$ = 6 Hz, 3H), 1.15−1.42 (m, 26 H), 1.61−1.63(m, 2H), 2.20 (t, $J$ = 8 Hz, 2H), 3.59 (t, $J$ = 6 Hz, 2H); $^{13}$C NMR ($CDCl_3$) $\delta$ (ppm) 14.10 ($CH_3$), 22.71, 23.35, 25.68, 29.37, 29.50, 29.69, 30.86, 31.96, 34.09, 36.75, 37.65, 41.21, 44.25 ($CH_2$), 173.39(C═O); mass spectrometry [M$^{+\bullet}$] = 318; IR $\nu$ (cm$^{-1}$) 3309 (NH), 1644(C═O). Anal. ($C_{18}H_{36}$-ClNO) C, H, N.

**Synthesis of *N*-(2-Bromoethyl)hexadecanamide (20).** The procedure described for compound **3** was used with 2 g (10 mmol) of 2-bromoethylamine hydrobromide, 3 mL of triethylamine (22 mmol), and 274 mg (1 mmol) of palmitoyl chloride to give 238 mg (66%) of a white solid: mp 83.6−86.0 °C (uncorrected); TLC (ethyl acetate/hexane 9:1 vv$^{-1}$) $R_f$ = 0.69; $^1$H NMR ($CDCl_3$) $\delta$ (ppm) 0.88 (t, $J$ = 6 Hz, 3H), 1.15−1.46 (m, 26 H), 1.61−1.63 (m, 2H), 2.20 (t, $J$ = 8 Hz, 2H), 3.59−3.62 (m, 2H); $^{13}$C NMR ($CDCl_3$) $\delta$ (ppm) 14.44 ($CH_3$), 26.08, 29.77, 29.89, 30.09, 33.00, 37.08, 41.61, 44.52 ($CH_2$), 173.79 (C═O); mass spectrometry [M$^{+\bullet}$] = 362; IR $\nu$ (cm$^{-1}$) 3309 (NH), 1644(C═O). Anal. ($C_{18}H_{36}BrNO$) C, H, N.

**Synthesis of *N*-(3-Chloropropyl)hexadecanamide (21).** The procedure described for compound **3** was used with 9.3 g (100 mmol) of 1-chloropropylamine and 2.74 g (10 mmol) of palmitoyl chloride to give 1.78 g (53%) of a white solid: mp 81.8−83.2 °C (uncorrected); TLC (ethyl acetate/hexane 9:1 vv$^{-1}$) $R_f$ = 0.6; $^1$H NMR ($CDCl_3$) $\delta$ (ppm) 0.88 (t, $J$ = 6 Hz, 3H), 1.15−1.35 (m, 26 H), 1.99 (t, $J$ = 7 Hz, 2H), 2.16 (t, $J$ = 7 Hz, 2H), 3.40−3.44 (m, 2H), 3.56−3.60 (m, 2H); $^{13}$C NMR ($CDCl_3$) $\delta$ (ppm) 14.10 ($CH_3$), 22.71, 25.75, 29.30, 29.50, 29.69, 31.89, 32.22, 36.81, 37.07, 42.57 ($CH_2$), 173.46 (C═O); mass spectrometry [M$^{+\bullet}$] = 332; IR $\nu$ (cm$^{-1}$) 3325 (NH), 1635(C═O). Anal. ($C_{19}H_{38}ClNO$) C, H, N.

**Aromatic Amides. Synthesis of *N*-Phenylhexadecanamide (22).** The procedure described for compound **3** was used with 9.3 g (100 mmol) of aniline and 2.74 g (10 mmol) of palmitoyl chloride to give 2.85 g (86%) of a white solid: mp 70.7−71.5 °C (uncorrected); TLC (chloroform) $R_f$ = 0.41; $^1$H NMR ($CDCl_3$) $\delta$ (ppm) 0.86 (t, $J$ = 7 Hz, 3H), 1.11−1.38 (m, 26 H), 2.33 (t, $J$ = 7 Hz, 2H), 7.06−7.09 (m, 1H), 7.25−7.32 (m, 2H), 7.32−7.52 (m, 2H); $^{13}$C NMR ($CDCl_3$) $\delta$ (ppm) 14.12 ($CH_3$), 22.71, 25.68, 29.31, 29.37, 29.41, 29.52, 29.71, 31.95, 37.86 ($CH_2$), 119.85, 124.16, 128.97, 138.04 (phenyl), 171.52 (C═O); mass spectrometry [M$^{+\bullet}$] = 332; IR $\nu$ (cm$^{-1}$) 3303 (NH), 1654 (C═O). CAS number: 6832-98-0.

**Synthesis of *N*-(4-Methylphenyl)hexadecanamide(23).** The procedure described for compound **3** was used with 10.7 g (100 mmol) of *p*-toluidine and 2.74 g (10 mmol) of palmitoyl chloride to give 2.7 g (78%) of a white solid: mp 95.6−96.7 °C (uncorrected); TLC (ethyl acetate/hexane 1:9 vv$^{-1}$) $R_f$ = 0.28; $^1$H NMR ($CDCl_3$) $\delta$ (ppm) 0.87 (t, $J$ = 7 Hz, 3H), 1.25−1.29 (m, 26 H), 2.30−2.34 (m, 5H), 7.19−7.25 (m, 2H), 7.37−7.39 (m, 2H); $^{13}$C NMR ($CDCl_3$) $\delta$ (ppm) 14.10 ($CH_3$), 20.83, 22.71, 25.69, 29.44, 29.69, 31.96, 37.85 ($CH_2$), 119.95, 129.46 (CH), 133.80, 135.48 (C), 171.32 (C═O); mass spectrometry [M$^{+\bullet}$] = 346; IR $\nu$ (cm$^{-1}$) 1661 (C═O). CAS number: 6876-53-5.

**Synthesis of *N*-(2-Methylphenyl)hexadecanamide (24).** The procedure described for compound **3** was used with 10.7 g (100 mmol) of *o*-toluidine and 2.74 g (10 mmol) of palmitoyl chloride to give 1.66 g (48%) of a white solid: mp 88.7−89.2 °C (uncorrected); TLC (ethyl acetate/hexane 1:9 vv$^{-1}$) $R_f$ = 0.22; $^1$H NMR ($CDCl_3$) $\delta$ (ppm) 0.86 (t, $J$ = 7 Hz, 3H), 1.25−1.29 (m, 26 H), 2.22 (s, $CH_3$), 2.35 (t, $J$ = 7 Hz, 2H), 7.00−7.02 (m, 1H), 7.04−7.06 (m, 1H), 7.16−7.18 (m, 1H), 7.23−7.25 (m, 1H); $^{13}$C NMR ($CDCl_3$) $\delta$ (ppm) 14.10, 17.79 ($CH_3$), 22.71, 24.84, 25.04, 25.88, 29.37, 29.69, 31.96, 37.65 ($CH_2$), 123.31, 125.13, 126.81, 129.01, 130.44, 135.81 (phenyl), 171.32 (C═O); mass spectrometry [M$^{+\bullet}$] = 346; IR $\nu$ (cm$^{-1}$) 1653 (C═O). CAS number: 54662−37−2

**Synthesis of 4′-Chlorohexadecananilide (25).** The procedure described for compound **3** was used with 12.7 g (100 mmol) of *p*-chloroaniline and 2.74 g (10 mmol) of palmitoyl chloride to give 1.83 g (50%) of a white solid: mp 98.2−99.6 °C (uncorrected); TLC (ethyl acetate/hexane 1:9 vv$^{-1}$) $R_f$ = 0.28; $^1$H NMR ($CDCl_3$) $\delta$ (ppm) 0.87 (t, $J$ = 7 Hz, 3H), 1.22−1.29 (m, 26 H), 2.35 (t, $J$ = 7 Hz, 2H), 7.19−7.27 (m, 2H), 7.44−7.47 (m, 2H); $^{13}$C NMR ($CDCl_3$) $\delta$ (ppm) 14.10 ($CH_3$), 22.71, 25.55, 29.37, 29.50, 29.69, 31.96, 37.78 ($CH_2$), 120.98, 129.01, 129.4, 136.58 (phenyl), 171.39 (C═O); mass spectrometry [M$^{+\bullet}$] = 366; IR $\nu$ (cm$^{-1}$) 1659 (C═O). CAS number: 100172-16-5.

**Synthesis of *N*-(1-Naphthalenyl)hexadecanamide (26).** The procedure described for compound **3** was used with 14.3 g (100 mmol) of 1-aminonaphthalene and 2.74 g (10 mmol) of palmitoyl chloride to give 1.98 g (52%) of a white solid: mp 110.8−112 °C (uncorrected); TLC (ethyl acetate/hexane 1:9 vv$^{-1}$) $R_f$ = 0.17; $^1$H NMR ($CDCl_3$) $\delta$ (ppm) 0.88 (t, $J$ = 7 Hz, 3H), 1.22−1.29 (m, 26 H), 2.52 (t, $J$ = 7 Hz, 2H), 7.25−7.87 (m, 7H); $^{13}$C NMR ($CDCl_3$) $\delta$ (ppm) 14.10 ($CH_3$), 22.70, 29.37, 29.50, 29.69, 31.96, 37.78 ($CH_2$), 120.98, 125.77, 126.14, 126.58, 127.64, 127.58, 128.24, 128.81, 129.01, 129.4 (naphthyl), 171.40 (C═O); mass spectrometry [M$^{+\bullet}$] = 382; IR $\nu$ (cm$^{-1}$) 1653 (C═O). CAS number: 79352-13-9.

**Synthesis of *N*-(Diphenylmethyl)hexadecanamide (27).** The procedure described for compound **3** was used with 18.3 g (100 mmol) of aminodiphenylmethane and 2.74 g (10 mmol) of palmitoyl chloride to give 1.94 g (46%) of a white solid: mp 116.3−118.2 °C (uncorrected); TLC (ethyl acetate/hexane 1:1 vv$^{-1}$) $R_f$ = 0.89; $^1$H NMR ($CDCl_3$) $\delta$ (ppm) 0.89 (t, $J$ = 7 Hz,

3H), 1.10−1.45 (m, 26 H), 2.23 (t, $J$ = 7 Hz, 2H), 6.04−6.08 (m, 1H), 7.19−7.34 (m, 10H); $^{13}$C NMR ($CDCl_3$) $\delta$ (ppm) 14.10 ($CH_3$), 22.70, 25.82, 29.37, 29.69, 31.96, 36.87 ($CH_2$), 56.80 (CH), 126.14, 127.45, 128.68, 141.75 (phenyls), 172.10 (C═O); mass spectrometry [$M^{+\bullet}$] = 422; IR $\nu$ ($cm^{-1}$) 3297 (NH), 1643 (C═O). CAS number: 10254-05-4.

**Synthesis of *N*-(4-Methylbenzyl)hexadecanamide (28).** The procedure described for compound **3** was used with 12.1 g (100 mmol) of *p*-methylbenzylamine and 2.74 g (10 mmol) of palmitoyl chloride to give 2.98 g (83%) of a white solid: mp 85.9−88.4 °C (uncorrected); TLC (ethyl acetate/hexane 4:6 $vv^{-1}$) $R_f$ = 0.46; $^1$H NMR ($CDCl_3$) $\delta$ (ppm) 0.88 (t, $J$ = 7 Hz, 3H), 1.15−1.35 (m, 26 H), 2.17 (s, $CH_3$), 2.28 (t, $J$ = 7 Hz, 2H), 4.39−4.41 (m, 2H), 7.13−7.17 (m, 2H), 7.24−7.28 (m, 2H); $^{13}$C NMR ($CDCl_3$) $\delta$ (ppm) 14.10, 14.23 ($CH_3$), 21.03, 22.70, 25.04, 25.82, 29.37, 29.50, 31.96, 34.03, 34.42, 36.81, 43.41, 60.10 ($CH_2$), 127.84, 128.56, 129.33, 135.48 (phenyl), 173.07 (C═O); mass spectrometry [$M^{+\bullet}$] = 360; IR $\nu$ ($cm^{-1}$) 3296 (NH), 1638 (C═O). Anal. ($C_{24}H_{41}NO$) C, H, N.

**Synthesis of *N*-(4-Methoxybenzyl)hexadecanamide (29).** The procedure described for compound **3** was used with 13.7 g (100 mmol) of *p*-methoxybenzylamine and 2.74 g (10 mmol) of palmitoyl chloride to give 3.08 g (82%) of a white solid: mp 81.8−82.3 °C (uncorrected); TLC (ethyl acetate/hexane 4:6 $vv^{-1}$) $R_f$ = 0.5; $^1$H NMR ($CDCl_3$) $\delta$ (ppm) 0.88 (t, $J$ = 7 Hz, 3H), 1.25−1.51 (m, 26 H), 2.16 (t, $J$ = 7 Hz, 2H), 3.85 (s, 3H), 4.42−4.44 (m, 2H), 6.88−6.94 (m, 2H), 7.26−7.28 (m, 2H); $^{13}$C NMR ($CDCl_3$) $\delta$ (ppm) 14.10 ($CH_3$), 22.71, 24.97, 25.81, 29.37, 29.69, 31.96, 34.03, 36.94, 39.33($CH_2$), 55.31 ($CH_3$), 110.37, 120.79 (CH), 128.81, 157.60 (C), 172.87 (C═O); mass spectrometry [$M^{+\bullet}$] = 376; IR $\nu$ ($cm^{-1}$) 3302 (NH), 1646 (C═O). Anal. ($C_{24}H_{41}NO_2$) C, H, N.

**Synthesis of *N*-Phenethylhexadecanamide (30).** The procedure described for compound **3** was used with 12.1 g (100 mmol) of phenethylamine and 2.74 g (10 mmol) of palmitoyl chloride to give 2.16 g (60%) of a white solid: mp 83.2−83.5 °C (uncorrected); TLC (ethyl acetate/methanol 8:2 $vv^{-1}$) $R_f$ = 0.83; $^1$H NMR ($CDCl_3$) $\delta$ (ppm) 0.87 (t, $J$ = 7 Hz, 3H), 1.15−1.35 (m, 26 H), 2.10 (t, $J$ = 7 Hz, 2H), 2.81 (t, $J$ = 7 Hz, 2H), 3.53 (t, $J$ = 7 Hz, 2H), 7.17−7.31 (m, 5H); $^{13}$C NMR ($CDCl_3$) $\delta$ (ppm) 14.10 ($CH_3$), 22.70, 25.75, 29.37, 29.50, 29.69, 31.96, 35.77, 36.87, 40.50 ($CH_2$), 126.55, 127.45, 128.62, 128.81 (phenyl), 173.07 (C═O); mass spectrometry [$M^{+\bullet}$] = 360; IR $\nu$ ($cm^{-1}$) 3316 (NH), 1638 (C═O). CAS number: 10015-69-7.

**Synthesis of *N*-(2-Hydroxyphenyl)hexadecanamide (31).** A solution of palmitoyl chloride (0.66 mmol) in 4 mL of dry benzene was cooled in an ice bath, and a solution of 2-aminophenol (6.6 mmol, 720 mg) in 9 mL of anhydrous THF was added dropwise. The reaction mixture was stirred at room temperature for 2 h and then diluted with 10 mL of methylene chloride and 10 mL of water. The organic layer was separated, and the solvents were removed under reduced pressure. The residue was chromatographed on silica gel (eluting with ethyl acetate/hexane 4:6 $vv^{-1}$) to give 150 mg (62%) of a white solid: mp 75.9−76.4 °C (uncorrected); TLC (ethyl acetate/hexane 4:6 $vv^{-1}$) $R_f$ = 0.79; $^1$H NMR (DMSO-$d_6$) $\delta$ (ppm) 0.85 (t, $J$ = 9 Hz, 3H), 1.16−1.28 (m, 26 H), 2.37 (t, $J$ = 7 Hz, 2H), 6.72−7.67 (m, 4H), 9.24 (s, 1H); $^{13}$C NMR (DMSO-$d_6$) $\delta$ (ppm) 13.75 ($CH_3$), 21.90, 25.07, 28.44, 28.63, 28.83, 31.09, 35.81 ($CH_2$), 115.98, 118.82, 122.12, 124.52 (CH), 126.26, 147.74 (C), 171.88 (C═O); mass spectrometry [$M^{+\bullet}$] = 347; IR $\nu$ ($cm^{-1}$) 3393 (NH), 3169 (OH), 1646 (C═O). Anal. ($C_{22}H_{37}NO_2$) C, H, N.

**Synthesis of *N*-(3-Hydroxyphenyl)hexadecanamide (32).** The procedure described for compound **31** was used with 720 mg (6.6 mmol) of 3-aminophenol and 0.66 mmol of palmitoyl chloride to give 130 mg (58%) of a white solid: mp 106.3−107 °C (uncorrected); TLC (ethyl acetate/hexane 4:6 $vv^{-1}$) $R_f$ = 0.6; $^1$H NMR (DMSO-$d_6$) $\delta$ (ppm) 0.83 (t, $J$ = 9 Hz, 3H), 1.16−1.34 (m, 26 H), 2.25 (t, $J$ = 7 Hz, 2H), 6.39−7.17 (m, 4H), 9.36 (s, 1H); $^{13}$C NMR (DMSO-$d_6$) $\delta$ (ppm) 13.84 ($CH_3$), 21.99, 25.10, 28.59, 28.72, 28.92, 31.18, 36.42 ($CH_2$), 106.23, 109.85, 109.98, 129.14 (CH), 140.33, 157.47 (C), 171.13 (C═O); mass spectrometry [$M^{+\bullet}$] = 347; IR $\nu$ ($cm^{-1}$) 3331 (NH), 3106 (OH), 1664 (C═O). Anal. ($C_{22}H_{37}NO_2$) C, H, N.

**Synthesis of *N*-(4-Hydroxyphenyl)hexadecanamide (33).** The procedure described for compound **31** was used with 720 mg (6.6 mmol) of 4-aminophenol and 0.66 mmol of palmitoyl chloride to give 164 mg (72%) of a white solid: mp 119.1−120 °C (uncorrected); TLC (ethyl acetate/hexane 4:6 $vv^{-1}$) $R_f$ = 0.54; $^1$H NMR (DMSO-$d_6$) $\delta$ (ppm) 0.86 (t, $J$ = 9 Hz, 3H), 1.16−1.42 (m, 26 H), 2.21 (t, $J$ = 7 Hz, 2H), 6.65 (d, 2H), 7.34 (d, 2H), 9.15 (s, 1H); $^{13}$C NMR (DMSO-$d_6$) $\delta$ (ppm) 14.40 ($CH_3$), 22.55, 25.66, 29.15, 29.47, 31.74, 36.72 ($CH_2$), 115.39, 121.28 (CH), 131.51, 153.50 (C), 170.97 (C═O); mass spectrometry [$M^{+\bullet}$] = 347; IR $\nu$ ($cm^{-1}$) 3315 (NH), 3070 (OH), 1653 (C═O). Anal. ($C_{22}H_{37}NO_2$) C, H, N.

**Synthesis of *N,N′*-Dimethylhexadecanamide (34).** The procedure described for compound **3** was used with 4.5 g (100 mmol) of dimethylamine and 2.74 g (10 mmol) of palmitoyl chloride to give 1.78 g (63%) of a white solid: mp 46−47 °C (uncorrected); TLC (chloroform/acetone 9:1 $vv^{-1}$) $R_f$ = 0.88; $^1$H NMR ($CDCl_3$) $\delta$ (ppm) 0.87 (t, $J$ = 7 Hz, 3H), 1.06−1.10 (m, 6H), 1.11−1.43 (m, 26 H), 2.43 (t, $J$ = 7 Hz, 2H); $^{13}$C NMR ($CDCl_3$) $\delta$ (ppm) 14.10, 14.12 ($CH_3$), 22.72, 24.29, 28.93, 29.24, 29.41, 29.45, 29.61, 29.72, 31.97, 35.34 ($CH_2$), 171.64 (C═O); mass spectrometry [$M^{+\bullet}$] = labile; IR $\nu$ ($cm^{-1}$) 1640 (C═O). CAS number: 3886-91-7.

**Synthesis of *N,N′*-Diethylhexadecanamide (35).** The procedure described for compound **3** was used with 7.3 g (100 mmol) of diethylamine and 2.74 g (10 mmol) of palmitoyl chloride to give 2.3 g (74%) of a yellowish oil: TLC (chloroform/methanol 6:4 $vv^{-1}$) $R_f$ = 0.82; $^1$H NMR ($CDCl_3$) $\delta$ (ppm) 0.87 (t, $J$ = 6 Hz, 3H), 1.09−1.16 (m, 6H), 1.22−1.41 (m, 26 H), 2.27 (t, $J$ = 7 Hz, 2H), 3.33 (q, $J$ = 12 Hz, 4H); $^{13}$C NMR ($CDCl_3$) $\delta$ (ppm) 13.18, 14.14 ($CH_3$), 22.79, 25.58, 29.64, 29.81, 32.04, 33.17, 40.11, 42.03 ($CH_2$), 172.11 (C═O); mass spectrometry [$M^{+\bullet}$] = 312; IR $\nu$ ($cm^{-1}$) 1646 (C═O). CAS number: 57303-21-6.

**Synthesis of 4-(1-Oxohexadecyl)morpholine (36).** The procedure described for compound **3** was used with 8.7 g (100 mmol) of morpholine and 2.74 g (10 mmol) of palmitoyl chloride to give 1.7 g (52%) of a white solid: mp 38−39 °C (uncorrected); TLC (chloroform/methanol 6:4 $vv^{-1}$) $R_f$ = 0.9; $^1$H NMR ($CDCl_3$) $\delta$ (ppm) 0.86 (t, $J$ = 6 Hz, 3H), 1.22−1.43 (m, 26 H), 2.29 (t, $J$ = 7 Hz, 2H), 3.43−3.46 (m, 4H), 3.62−3.65 (m, 4H); $^{13}$C NMR ($CDCl_3$) $\delta$ (ppm) 14.12 ($CH_3$), 22.71, 25.02, 25.31, 29.39, 29.47, 29.52, 29.55, 29.69, 31.73, 31.96, 33.14, 41.92, 46.13, 66.72 ($CH_2$), 171.89 (C═O); mass spectrometry [$M^{+\bullet}$] = 326; IR $\nu$ ($cm^{-1}$) 1649 (C═O). CAS number: 5299-68-3.

**Synthesis of *N*-(2-Acetoxyacetyl)pentadecylamine (37).** In a two-neck flask, an amount of 500 mg (2.2 mmol) of pentadecylamine was poured into a solution of 10 mL of dry methylene chloride with 300 $\mu$L of pyridine. Acetoxyacetyl chloride (395 mg, 2.9 mmol) was added dropwise at room temperature. The reaction mixture was stirred for 1 h. Excess acetoxyacetyl chloride was destroyed by adding 350 $\mu$L of water. The mixture was then washed with 10% citric acid solution, 5% bicarbonate solution, and brine. The solvent was evaporated under reduced pressure to afford 618 mg (86%) of a white solid: mp 69.8−70.2 °C (uncorrected); TLC (ethyl acetate/dichloromethane 1:9 $vv^{-1}$) $R_f$ = 0.42; $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ (ppm) 0.86 (t, $J$ = 6 Hz, 3H), 1.10−1.41 (m, 26 H), 2.16 (s, 3H), 3.26−3.31 (m, 2H), 4.54 (s, 2H); $^{13}$C NMR (300 MHz, $CDCl_3$) $\delta$ (ppm) 14.1, 20.70 ($CH_3$), 22.71, 26.85, 29.24, 29.37, 29.56, 29.69, 31.96, 39.27, 63.21 ($CH_2$), 166.79, 169.32 (C═O); mass spectrometry [$M^{+\bullet}$] = 328; IR $\nu$ ($cm^{-1}$) 3306 (NH), 1761, 1655 (C═O). Anal. ($C_{19}H_{37}NO_3$) C, H, N.

**Pharmacology. Compounds.** Anandamide [ethanolamine-1-$^3$H] ([$^3$H]-AEA, specific activity of 60 Ci $mmol^{-1}$) was obtained from American Radiolabeled Chemicals, Inc. (St. Louis, MO). [$^3$H]-WIN 55,212-2 (specific activity of 45.5 Ci $mmol^{-1}$) and [$^3$H]-CP55,940 (specific activity of 101.1 Ci $mmol^{-1}$) were obtained from New England Nuclear, Boston, MA. Nonradioactive anandamide (arachidonoylethanolamide, AEA) was purchased from the Cayman Chemical Company (Ann Arbor, MI). Fatty acid free bovine serum albumin (BSA) was obtained

from Sigma Chemical Co. (St. Louis, MO). All cell culture media, sera, and supplements were from Gibco/Life Technologies.

**Membrane Preparation for FAAH Assay.** Frozen brains (minus cerebellum) stored at −70 °C from adult rats were thawed and homogenized at 4 °C in 20 mM HEPES buffer, pH 7.0, with 1 mM $MgCl_2$ using a glass homogenizer. The homogenates were centrifuged twice (36000*g* for 20 min at 4 °C) after which the tissue pellets were resuspended in homogenization buffer and incubated at 37 °C for 15 min. After centrifugation at 36000*g* for 20 min at 4 °C, membranes were resuspended in 50 mM Tris-HCl buffer, pH 7.4, containing 1 mM EDTA and 3 mM $MgCl_2$. Protein content in the membrane preparations was determined according to the method of Harrington,[51] using bovine serum albumin as standard, and was stored at −70 °C until used for the assay.

**FAAH Assay.** The method, adapted to the tritiated substrate as described previously,[52] was based on that of Omeir et al.[53] who used $^{14}$C-labeled AEA as substrate. Membranes, test compounds or ethanol carrier (10 $\mu$L), [$^3$H]-AEA (2 $\mu$M final concentration), and assay buffer (10 mM Tris-HCl, 1 mM EDTA, 1% (w/v) BSA, pH 7.6) were incubated at 37 °C for 10 min. Reactions were stopped by placing the tubes in ice and adding 400 $\mu$L of chloroform/methanol (1:1 v/v) followed by vortex mixing. Phases were separated by centrifugation in a bench centrifuge, and aliquots (200 $\mu$L) of the methanol/buffer phase were analyzed for radioactivity by liquid scintillation spectroscopy with quench correction. Blanks contained distilled water instead of the homogenate preparations.

**Radioligand Binding Experiments.** Human $CB_1$- ("CHO-$CB_1$") and $CB_2$- ("CHO-$CB_2$") transfected CHO cells, kindly donated by Drs. Nokin and Detheux (Euroscreen, Belgium), were maintained in culture using Ham's F12 medium containing 10% fetal bovine serum, 100 $\mu$g/mL streptomycin, 100 U/mL penicillin, and 200 $\mu$g/mL G418. Membranes (40 $\mu$g) were incubated at 30 °C with 1 nM [$^3$H]-CP55,940 (CHO-$CB_1$) or 1 nM [$^3$H]-WIN 55,212−2 (CHO-$CB_2$) for 1 h in 50 mM Tris-HCl with $MgCl_2$ and EDTA (pH 7.4) in the presence of 50 $\mu$M PMSF. Nonspecific binding was determined with 10 $\mu$M HU-210 (CHO-$CB_1$). After incubation, the membrane suspension was rapidly filtered through 0.5% polyethyleneimine-pretreated GF/B glass fiber filters (Whatman), and the radioactivity trapped on the filters was measured by liquid scintillation spectroscopy. Assays were undertaken in quadruplicate.

**Acknowledgment.** The authors thank Britt Jacobsson for excellent technical assistance with the FAAH assays. The help of Professor G. K. E. Scriba of University of Iena, Germany, with the mass spectra is gratefully acknowledged. This study was supported by grants from the Belgian National Fund for Scientific Research, an FSR grant from the Université Catholique de Louvain, the Charcot Foundation "Groupe Belge d'Etude de la Sclérose en Plaques", the Swedish Research Council (Grant No. 12158, Medicine), the Swedish Asthma and Allergy Association's Research Foundation, Konung Gustav V's and Drottning Victorias Foundation, the Swedish Psoriasis Association, and the Research Funds of the Medical Odontological Faculty, Umeå University.

## References

(1) Devane, W. A.; Hanus, L.; Breuer, A.; Pertwee, R. G.; Stevenson, L. A.; Griffin, G.; Gibson, D.; Mandelbaum, A.; Etinger, A.; Mechoulam, R. Isolation and Structure of a Brain Constituent That Binds to the Cannabinoid Receptor. *Science* **1992**, *258*, 1946−1949.

(2) Mechoulam, R.; Fride, E.; Di Marzo, V. Endocannabinoids. *Eur. J. Pharmacol.* **1998**, *359*, 1−18.

(3) Calignano, A.; La Rana, G.; Giuffrida, A.; Piomelli, D. Control of Pain Initiation by Endogenous Cannabinoids. *Nature* **1998**, *394*, 277−281.

(4) Jaggar, S. I.; Hasnie, F. S.; Sellaturay, S.; Rice, A. S. The Anti-Hyperalgesic Actions of the Cannabinoid Anandamide and the Putative CB2 Receptor Agonist Palmitoylethanolamide in Visceral and Somatic Inflammatory Pain. *Pain* **1998**, *76*, 189−199.

(5) Richardson, J. D.; Aanonsen, L.; Hargreaves, K. M. Antihyperalgesic Effects of Spinal Cannabinoids. *Eur. J. Pharmacol.* **1998**, *345*, 145−153.

(6) Raffa, R. B.; Stone, J.; Hipp, S. J. Differential Cholera-Toxin Sensitivity of Supraspinal Antinociception Induced by the Cannabinoid Agonists [Delta]9-THC, WIN 55,212-2 and Anandamide in Mice. *Neurosci. Lett.* **1999**, *263*, 29−32.

(7) Gauldie, S. D.; McQueen, D. S.; Pertwee, R.; Chessell, I. P. Anandamide Activates Peripheral Nociceptors in Normal and Arthritic Rat Knee Joints. *Br. J. Pharmacol.* **2001**, *132*, 617−621.

(8) Terranova, J. P.; Michaud, J. C.; Le Fur, G.; Soubrie, P. Inhibition of Long-Term Potentiation in Rat Hippocampal Slices by Anandamide and WIN55212-2: Reversal by SR141716 A, a Selective Antagonist of CB1 Cannabinoid Receptors. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1995**, *352*, 576−579.

(9) Mallet, P. E.; Beninger, R. J. Delta9-Tetrahydrocannabinol, but Not the Endogenous Cannabinoid Receptor Ligand Anandamide, Produces Conditioned Place Avoidance. *Life Sci.* **1998**, *62*, 2431−2439.

(10) Calignano, A.; Katona, I.; Desarnaud, F.; Giuffrida, A.; La Rana, G.; Mackie, K.; Freund, T. F.; Piomelli, D. Bidirectional Control of Airway Responsiveness by Endogenous Cannabinoids. *Nature* **2000**, *408*, 96−101.

(11) Baker, D.; Pryce, G.; Croxford, J. L.; Brown, P.; Pertwee, R. G.; Makriyannis, A.; Khanolkar, A.; Layward, L.; Fezza, F.; Bisogno, T.; Di Marzo, V. Endocannabinoids Control Spasticity in a Multiple Sclerosis Model. *FASEB J.* **2001**, *15*, 300−302.

(12) De Petrocellis, L.; Melck, D.; Palmisano, A.; Bisogno, T.; Laezza, C.; Bifulco, M.; Di Marzo, V. The Endogenous Cannabinoid Anandamide Inhibits Human Breast Cancer Cell Proliferation. *Proc. Natl. Acad. Sci. U.S.A* **1998**, *95*, 8375−8380.

(13) Sarker, K. P.; Obara, S.; Nakata, M.; Kitajima, I.; Maruyama, I. Anandamide Induces Apoptosis of PC-12 Cells: Involvement of Superoxide and Caspase-3. *FEBS Lett.* **2000**, *472*, 39−44.

(14) Piomelli, D.; Giuffrida, A.; Calignano, A.; de Fonseca, F. R. The Endocannabinoid System As a Target for Therapeutic Drugs. *Trends Pharmacol. Sci.* **2000**, *21*, 218−224.

(15) Di Marzo, V.; Bisogno, T.; De Petrocellis, L. Endocannabinoids: New Targets for Drug Development. *Curr. Pharm. Des.* **2000**, *6*, 1361−1380.

(16) Zygmunt, P. M.; Petersson, J.; Andersson, D. A.; Chuang, H.; Sorgard, M.; Di Marzo, V.; Julius, D.; Hogestatt, E. D. Vanilloid Receptors on Sensory Nerves Mediate the Vasodilator Action of Anandamide. *Nature* **1999**, *400*, 452−457.

(17) Smart, D.; Gunthorpe, M. J.; Jerman, J. C.; Nasir, S.; Gray, J.; Muir, A. I.; Chambers, J. K.; Randall, A. D.; Davis, J. B. The Endogenous Lipid Anandamide Is a Full Agonist at the Human Vanilloid Receptor (HVR1). *Br. J. Pharmacol.* **2000**, *129*, 227−230.

(18) Deutsch, D. G.; Chin, S. A. Enzymatic Synthesis and Degradation of Anandamide, a Cannabinoid Receptor Agonist. *Biochem. Pharmacol.* **1993**, *46*, 791−796.

(19) Rodriguez De Fonseca, F.; Navarro, M.; Gomez, R.; Escuredo, L.; Nava, F.; Fu, J.; Murillo-Rodriguez, E.; Giuffrida, A.; LoVerme, J.; Gaetani, S.; Kathuria, S.; Gall, C.; Piomelli, D. An Anorexic Lipid Mediator Regulated by Feeding. *Nature* **2001**, *414*, 209−212.

(20) Natarajan, V.; Schmid, P. C.; Schmid, H. H. *N*-Acylethanolamine Phospholipid Metabolism in Normal and Ischemic Rat Brain. *Biochim. Biophys. Acta* **1986**, *878*, 32−41.

(21) Cravatt, B. F.; Giang, D. K.; Mayfield, S. P.; Boger, D. L.; Lerner, R. A.; Gilula, N. B. Molecular Characterization of an Enzyme That Degrades Neuromodulatory Fatty-Acid Amides. *Nature* **1996**, *384*, 83−87.

(22) Schmid, P. C.; Zuzarte-Augustin, M. L.; Schmid, H. H. Properties of Rat Liver *N*-Acylethanolamine Amidohydrolase. *J. Biol. Chem.* **1985**, *260*, 14145−14149.

(23) Fowler, C. J.; Jonsson, K. O.; Tiger, G. Fatty Acid Amide Hydrolase: Biochemistry, Pharmacology, and Therapeutic Possibilities for an Enzyme Hydrolyzing Anandamide, 2-Arachidonoylglycerol, Palmitoylethanolamide, and Oleamide. *Biochem. Pharmacol.* **2001**, *62*, 517−526.

(24) Childers, S. R.; Sexton, T.; Roy, M. B. Effects of Anandamide on Cannabinoid Receptors in Rat Brain Membranes. *Biochem. Pharmacol.* **1994**, *47*, 711−715.

(25) Gifford, A. N.; Bruneus, M.; Lin, S.; Goutopoulos, A.; Makriyannis, A.; Volkow, N. D.; Gatley, S. J. Potentiation of the Action of Anandamide on Hippocampal Slices by the Fatty Acid Amide Hydrolase Inhibitor, Palmitylsulphonyl Fluoride (AM 374). *Eur. J. Pharmacol.* **1999**, *383*, 9−14.

(26) Pertwee, R. G.; Fernando, S. R.; Griffin, G.; Abadji, V.; Makriyannis, A. Effect of Phenylmethylsulphonyl Fluoride on the Potency of Anandamide As an Inhibitor of Electrically Evoked Contractions in Two Isolated Tissue Preparations. *Eur. J. Pharmacol.* **1995**, *272*, 73−78.
(27) Cravatt, B. F.; Demarest, K.; Patricelli, M. P.; Bracey, M. H.; Giang, D. K.; Martin, B. R.; Lichtman, A. H. Supersensitivity to Anandamide and Enhanced Endogenous Cannabinoid Signaling in Mice Lacking Fatty Acid Amide Hydrolase. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 9371−9376.
(28) Bracey, M. H.; Hanson, M. A.; Masuda, K. R.; Stevens, R. C.; Cravatt, B. F. Structural Adaptations in a Membrane Enzyme That Terminates Endocannabinoid Signaling. *Science* **2002**, *298*, 1793−1796.
(29) Bisogno, T.; Melck, D.; De Petrocellis, L.; Bobrov, M. Y.; Gretskaya, N. M.; Bezuglov, V. V.; Sitachitta, N.; Gerwick, W. H.; Di Marzo, V. Arachidonoylserotonin and Other Novel Inhibitors of Fatty Acid Amide Hydrolase. *Biochem. Biophys. Res. Commun.* **1998**, *248*, 515−522.
(30) Koutek, B.; Prestwich, G. D.; Howlett, A. C.; Chin, S. A.; Salehani, D.; Akhavan, N.; Deutsch, D. G. Inhibitors of Arachidonoyl Ethanolamide Hydrolysis. *J. Biol. Chem.* **1994**, *269*, 22937−22940.
(31) Boger, D. L.; Fecik, R. A.; Patterson, J. E.; Miyauchi, H.; Patricelli, M. P.; Cravatt, B. F. Fatty Acid Amide Hydrolase Substrate Specificity. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2613−2616.
(32) Bisogno, T.; Maurelli, S.; Melck, D.; De Petrocellis, L.; Di Marzo, V. Biosynthesis, Uptake, and Degradation of Anandamide and Palmitoylethanolamide in Leukocytes. *J. Biol. Chem.* **1997**, *272*, 3315−3323.
(33) Lambert, D. M.; DiPaolo, F. G.; Sonveaux, P.; Kanyonyo, M.; Govaerts, S. J.; Hermans, E.; Bueb, J.; Delzenne, N. M.; Tschirhart, E. J. Analogues and Homologues of *N*-Palmitoylethanolamide, a Putative Endogenous CB(2) Cannabinoid, As Potential Ligands for the Cannabinoid Receptors. *Biochim. Biophys. Acta* **1999**, *1440*, 266−274.
(34) Lambert, D. M.; Di Marzo, V. The Palmitoylethanolamide and Oleamide Enigmas: Are These Two Fatty Acid Amides Cannabimimetic? *Curr. Med. Chem.* **1999**, *6*, 757−773.
(35) Lambert, D. M.; Vandevoorde, S.; Jonsson, K. O.; Fowler, C. J. The Palmitoylethanolamide Family: A New Class of Anti-Inflammatory Agents? *Curr. Med. Chem.* **2002**, *9*, 663−674.
(36) Deutsch, D. G.; Lin, S.; Hill, W. A.; Morse, K. L.; Salehani, D.; Arreaza, G.; Omeir, R. L.; Makriyannis, A. Fatty Acid Sulfonyl Fluorides Inhibit Anandamide Metabolism and Bind to the Cannabinoid Receptor. *Biochem. Biophys. Res. Commun.* **1997**, *231*, 217−221.
(37) Jonsson, K. O.; Vandevoorde, S.; Lambert, D. M.; Tiger, G.; Fowler, C. J. Effects of Homologues and Analogues of Palmitoylethanolamide Upon the Inactivation of the Endocannabinoid Anandamide. *Br. J. Pharmacol.* **2001**, *133*, 1263−1275.
(38) Smart, D.; Jonsson, K. O.; Vandevoorde, S.; Lambert, D. M.; Fowler, C. J. "Entourage" Effects of *N*-Acyl Ethanolamines at Human Vanilloid Receptors. Comparison of Effects Upon Anandamide-Induced Vanilloid Receptor Activation and Upon Anandamide Metabolism. *Br. J. Pharmacol.* **2002**, *136*, 452−458.
(39) De Petrocellis, L.; Davis, J. B.; Di Marzo, V. Palmitoylethanolamide Enhances Anandamide Stimulation of Human Vanilloid VR1 Receptors. *FEBS Lett.* **2001**, *506*, 253−256.
(40) Ben Shabat, S.; Fride, E.; Sheskin, T.; Tamiri, T.; Rhee, M. H.; Vogel, Z.; Bisogno, T.; De Petrocellis, L.; Di Marzo, V.; Mechoulam, R. An Entourage Effect: Inactive Endogenous Fatty Acid Glycerol Esters Enhance 2-Arachidonoyl-Glycerol Cannabinoid Activity. *Eur. J. Pharmacol.* **1998**, *353*, 23−31.
(41) Tiger, G.; Stenstrom, A.; Fowler, C. J. Pharmacological Properties of Rat Brain Fatty Acid Amidohydrolase in Different Subcellular Fractions Using Palmitoylethanolamide As Substrate. *Biochem. Pharmacol.* **2000**, *59*, 647−653.
(42) Jarrahian, A.; Manna, S.; Edgemond, W. S.; Campbell, W. B.; Hillard, C. J. Structure−Activity Relationships Among *N*-Arachidonylethanolamine (Anandamide) Head Group Analogues for the Anandamide Transporter. *J. Neurochem.* **2000**, *74*, 2597−2606.
(43) Lang, W.; Qin, C.; Lin, S.; Khanolkar, A. D.; Goutopoulos, A.; Fan, P.; Abouzid, K.; Meng, Z.; Biegel, D.; Makriyannis, A. Substrate Specificity and Stereoselectivity of Rat Brain Microsomal Anandamide Amidohydrolase. *J. Med. Chem.* **1999**, *42*, 896−902.
(44) Pinto, J. C.; Potie, F.; Rice, K. C.; Boring, D.; Johnson, M. R.; Evans, D. M.; Wilken, G. H.; Cantrell, C. H.; Howlett, A. C. Cannabinoid Receptor Binding and Agonist Activity of Amides and Esters of Arachidonic Acid. *Mol. Pharmacol.* **1994**, *46*, 516−522.
(45) Sheskin, T.; Hanus, L.; Slager, J.; Vogel, Z.; Mechoulam, R. Structural Requirements for Binding of Anandamide-Type Compounds to the Brain Cannabinoid Receptor. *J. Med. Chem.* **1997**, *40*, 659−667.
(46) Bisogno, T.; Melck, D.; Bobrov, M. Y.; Gretskaya, N. M.; Bezuglov, V. V.; De Petrocellis, L.; Di Marzo, V. *N*-Acyl-Dopamines: Novel Synthetic CB1 Cannabinoid-Receptor Ligands and Inhibitors of Anandamide Inactivation With Cannabimimetic Activity in Vitro and in Vivo. *Biochem. J.* **2000**, *351*, 817−824.
(47) Holt, S.; Nilsson, J.; Omeir, R.; Tiger, G.; Fowler, C. J. Effects of pH on the Inhibition of Fatty Acid Amidohydrolase by Ibuprofen. *Br. J. Pharmacol.* **2001**, *133*, 513−520.
(48) Boger, D. L.; Sato, H.; Lerner, A. E.; Austin, B. J.; Patterson, J. E.; Patricelli, M. P.; Cravatt, B. F. Trifluoromethyl Ketone Inhibitors of Fatty Acid Amide Hydrolase: A Probe of Structural and Conformational Features Contributing to Inhibition. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 265−270.
(49) Lambert, D. M.; Vandevoorde, S.; Diependaele, G.; Govaerts, S. J.; Robert, A. R. Anticonvulsant Activity of *N*-Palmitoylethanolamide, a Putative Endocannabinoid, in Mice. *Epilepsia* **2001**, *42*, 321−327.
(50) Ikeda, M.; Kusaka, T. Liquid chromatography mass spectrometry of hydroxy and non hydroxy fatty acids as amide derivatives. *J. Chromatogr.* **1992**, *575*, 197−205.
(51) Harrington, C. R. Lowry Protein Assay Containing Sodium Dodecyl Sulfate in Microtiter Plates for Protein Determinations on Fractions From Brain Tissue. *Anal. Biochem.* **1990**, *186*, 285−287.
(52) Fowler, C. J.; Tiger, G.; Strenström, A. Ibuprofen Inhibits Rat Brain Deamidation of Anandamide at Pharmacologically Relevant Concentrations. Mode of Inhibition and Structure−Activity Relationship. *J. Pharmacol. Exp. Ther.* **1997**, *283*, 729−734.
(53) Omeir, R. L.; Chin, S.; Hong, Y.; Ahern, D. G.; Deutsch, D. G. Arachidonoyl Ethanolamide-[1,2-14C] As a Substrate for Anandamide Amidase. *Life Sci.* **1995**, *56*, 1999−2005.

JM0209679