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Design, Synthesis, and Structure–Activity Relationships of Alkylcarbamic Acid Aryl Esters, a New Class of Fatty Acid Amide Hydrolase Inhibitors

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Fatty acid amide hydrolase (FAAH), an intracellular serine hydrolase enzyme, participates in the deactivation of fatty acid ethanolamides such as the endogenous cannabinoid anandamide, the intestinal satiety factor oleoylethanolamide, and the peripheral analgesic and anti-inflammatory factor palmitoylethanolamide. In the present study, we report on the design, synthesis, and structure–activity relationships (SAR) of a novel class of potent, selective, and systemically active inhibitors of FAAH activity, which we have recently shown to exert potent anxiolytic-like effects in rats. These compounds are characterized by a carbamic template substituted with alkyl or aryl groups at their O- and N-termini. Most compounds inhibit FAAH, but not several other serine hydrolases, with potencies that depend on the size and shape of the substituents. Initial SAR investigations suggested that the requirements for optimal potency are a lipophilic N-alkyl substituent (such as n-butyl or cyclohexyl) and a bent O-aryl substituent. Furthermore, the carbamic group is essential for activity. A 3D-QSAR analysis on the alkylcarbamic acid aryl esters showed that the size and shape of the O-aryl moiety are correlated with FAAH inhibitory potency. A CoMSIA model was constructed, indicating that whereas the steric occupation of an area corresponding to the meta position of an O-phenyl ring improves potency, a region of low steric tolerance on the enzyme active site exists corresponding to the para position of the same ring. The bent shape of the O-aryl moieties that best fit the enzyme surface closely resembles the folded conformations observed in the complexes of unsaturated fatty acids with different proteins. URB524 (N-cyclohexylcarbamic acid biphenyl-3-yl ester, 9g) is the most potent compound of the series (IC$_{50}$ = 63 nM) and was therefore selected for further optimization.

Introduction

The fatty acid ethanolamides (FAEs) constitute a newly characterized family of lipid mediators,† which include the endogenous cannabinoid (endocannabinoid) anandamide (arachidonyl ethanolamide, 1, Figure 1), the intestinal satiety factor oleoyl ethanolamide (OEA, 2, Figure 1), and the peripheral analgesic and anti-inflammatory factor palmitoylethanolamide (PEA, 3, Figure 1). The FAEs are normally present in mammalian organs and tissues, where their formation is thought to be mediated through receptor-dependent cleavage of N-acylphosphatidylethanolamine (NAPE), a minor phospholipid component of cell membranes. After formation and release from cells, polyunsaturated FAEs such as anandamide may be eliminated via a two-step process consisting of high-affinity transport into cells, followed by intracellular degradation catalyzed by the serine hydrolase, fatty acid amide hydrolase (FAAH). On the other hand, saturated and monounsaturated FAEs such as OEA and PEA are not substrates for transmembrane transport and their inactiva-
among which 1-oxazolo[4,5-b]pyridin-2-yl ketones substituted with a C8–C12 unsaturated chain or with a phenylalkyl chain of four to eight methylene units (e.g., compound 5, Figure 2) gave the best results. Reversible inhibitors comprise fatty acid sulfonyl fluorides or fluorophosphonates such as hexadecanesulfonyl fluoride (AM374, 6, Figure 2).

Although the pharmacological properties of anandamide and other FAAEs underscore the potential therapeutic interest of FAAH inhibition, the development of suitable clinical candidates from currently available inhibitors may be difficult. The presence of a lipid-like chain may confer to these compounds a number of unfavorable biopharmaceutical properties, including low water solubility, high plasma protein binding, and high accumulation in fat tissues. Furthermore, the alkyl chain also may limit the target selectivity of current FAAH inhibitors by increasing their tendency to bind to other endocannabinoid-metabolizing enzymes, such as monoglyceride lipase and cannabinoid receptors. Therefore, the design of potent and selective small-molecule inhibitors of FAAH remains an essential step in the validation of this enzyme as a therapeutic target.

The cloning of rodent and human FAAH has revealed the existence of a close structural relationship among different mammalian forms of this enzyme and between these and bacterial amidases. This homology is particularly evident at the level of the amidase “signature sequence”, a highly conserved domain enriched in glycine and serine residues. Site-directed mutagenesis studies have shown that three serine residues within this domain are essential for catalytic activity (S217, S218, and S241), one of which (S241) may act as nucleophile during the catalytic process. This nucleophilic serine may be activated by the lysine residue K142 rather than through the Ser-His-Asp interaction characteristic of other serine hydrolases.

In the present study, we report on the synthesis of a series of alkylcarbamic acid aryl esters (9), designed as inhibitors of FAAH by progressive modification of the structure of a known inhibitor of the serine hydrolase acetylcholinesterase (AChE) (compound 19), and on the biological activity of some other known carbamates (compounds 7, 20–27 in Table 1). We investigated the importance for FAAH inhibition of the carbamate group and the stereoelectronic properties of its N- and the O-substituents. In particular, we evaluated the role of the carbamate group by replacing it with isoster groups. Moreover, carbamic acid esters having confor-
The synthesis of compounds 9a–i were obtained by addition of the suitable arylic aryl alcohols to isocyanato cyclohexane. Carbamate 11 was prepared by treatment of diimidazol-1-ylmethanone (10) with biphényl-3-ylamine, followed by cyclohexanol, and amide 13 from naphthalene-2-carboxylic acid (12) via acyl chloride. The preparation of 16a–d involved coupling of an iso(thio)cyanate with an appropriate thiol or amine. Finally, the thiocarbamate 18 was synthesized from thiophosgene (17), naphthalene-2-ol, and cyclohexylamine.

Compounds 7 and 19–27 were purchased from Sigma-Aldrich.

Results and Discussion
We measured FAAH activity in rat brain membranes, using [3H]anandamide as a substrate. Half-maximal concentrations (IC50) for inhibition of FAAH activity by compounds 9a–i, 11, 13, 16a–d, and 19–27 are reported in Table 1. We first tested the AChE inhibitor carbaryl (19) and its p-tolyl (20) and 2-naphthyl (21) analogues. Only the latter analogue showed a weak inhibitory activity, which was enhanced by introducing more lipophilic alkyl residues on the carbamic nitrogen. Thus, replacing the methyl group of 21 with a cyclohexyl resulted in a 60-fold decrease in IC50 (compound 22). Submicromolar potencies also were observed with the n-butyl derivatives 7 and 23, whereas the very low activity of 24 confirmed the essential role of the O-substituent in FAAH inhibition. Importantly, unlike carbaryl (19), which inhibited eel acetylcholinesterase (AChE) activity with an IC50 of 3.1 μM, compounds 7 and 20–24 had no effect on the activities of this enzyme or of horse-serum butyrylcholinesterase when tested at concentrations as high as 30 μM (data not shown). Furthermore, the compounds (1–100 μM) did not displace the binding of [3H]WIN-55212-2 (10 nM) to rat cerebellar CB1 receptors or human recombinant CB2 receptors and did not affect [3H]anandamide (100 nM) transport in astrocytoma cells (data not shown).

Next, to examine the role of the carbamate function in FAAH inhibition, we prepared several isosters of 22. Both the ester 13 and the urea 16a were ineffective. Furthermore, replacement of oxygen with sulfur (compounds 16b–d, 18) also led to inactive analogues, with the partial exception of the thiocarbamic acid S-naphthyl ester 16b. These results suggest that the carbamic group is essential for inhibition of FAAH activity by this series of compounds.

The hypothesis that guided our subsequent design was that the FAAH inhibitory activity may be improved by introducing aromatic substituents of defined shape on the oxygen atom. We further assumed that this moiety acted as a leaving group after the nucleophilic attack of the S241 residue of FAAH on the carbonyl of the inhibitors, resulting in enzyme carbamylation. Though direct evidence for this mechanism is still needed, the findings that compound 7 inhibits FAAH activity in a noncompetitive manner (Figure 4) and that extensive dialysis (1 L/mL of sample, 16 h at 4 °C) does not reverse inhibition (data not shown) support the notion that these compounds interact irreversibly with FAAH.

Comparison of the structures of compounds 7 and 22 provided a first insight into the shape requirements for the O-substituent. A systematic conformational analysis

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**Scheme 1**

a Reagents and conditions: (a) c-C6H11Cl, NCO, Et3N, toluene, reflux, 8–24 h; (b) biphényl-3-ylamine, CH3CN, DMAP, reflux, 12 h; (b) c-C6H11OH, reflux, 24 h; (c) (COCl)2, THF, 25 °C, 2 h; (d) c-C6H11NH2, Et3N, CH2Cl2, 25 °C, 20 h; (e) Et3N, toluene, reflux, 20 h for 16a; t-BuOK, toluene, 50 °C, 15 min for 16b; CH2Cl2, reflux, 14 h for 16c; 80–100 °C, 20 h for 16d; (f) sodium naphthalen-2-date, CHCl3, 25 °C, 1 h; (f) c-C6H11NH2, 25 °C, 4 h.

mationally constrained O-aryl moieties, designed to mimic the binding conformation of the FAEs chain, allowed us to conduct a 3D-QSAR analysis of the steric requirements of the carbamate group O-substituent.

We have recently shown that representative members of this series (e.g., URB532, 7, Figure 3) are systemically active inhibitors of FAAH activity. The fact that these compounds exert profound anxiolytic-like effects in rats suggests that they may provide candidates for the development of innovative antianxiety medicines.
of the benzylxoyphenyl fragment of 7 revealed two families of accessible conformers, mainly differing in the torsion angle around the O–CH$_2$ bond, with the two phenyl rings in anti or in gauche conformation. Apparently, the gauche conformation of 7 more closely resembled the shape of the naphthyl derivative 23 when the compounds were superimposed via their common carbamate group (Figure 5A). This led us to hypothesize that a bent shape of the carbamate O-substituent could favor enzyme inhibition, possibly by allowing a greater steric complementarity between the inhibitor and the active site of the enzyme. This hypothesis was further developed by supposing that the aryloxy group may mimic the fatty acid chain of anandamide and other FAEs (e.g., 1–3) by counterfeiting the arrangement of the first 10–12 carbon atoms in the so-called “U-shaped” conformation of these molecules.20

Accordingly, we conducted a systematic exploration of the steric requirements of the aromatic O-substituents by synthesizing a series of carbamates in which the shape of the O-residue was gradually modified, while maintaining the cyclohexyl substituent on the carbamic nitrogen. Starting from the phenyl and naphthyl derivatives 9a and 22, we prepared a series of compounds with lipophilic groups potentially able to occupy either a region of space antipodal to the carbamate group (9e, 25, 26) or a region corresponding to the meta position of the phenyl ring (9a–d, f–g). The design of these compounds took into consideration the possibility of calculating a 3D-QSAR model correlating steric parameters with inhibitory potency after superposition of their modeled structures.

Within this second set of compounds, greater inhibitory potencies were obtained with those molecules that had a bent shape. In particular, we observed the strongest FAAH inhibition with the m-biphenyl derivative URB524 (9g), whose IC$_{50}$ value (63 nM) indicates a 36-fold increase in potency over the isomeric p-biphenyl derivative 26. The comparison between 9e and 9f, 9c and 9d, and 9b and 25 is also suggestive of a similar trend. While the O-phenyl derivative 9a was almost inactive, we obtained compounds of moderate to good inhibitory potency by substituting the meta position of 9a with groups of suitable sizes (9b, h, g). Importantly, the carbamates 9a–g, 25, and 26 did not significantly interact with AChE or with cannabinoid-related targets such as CB$_1$ or CB$_2$ cannabinoid receptors and endocannabinoid transporter at concentrations as high as 30 µM.

It is noteworthy that the m-biphenyl fragment of 9g may be superposed to the first 10 carbons of the fatty acid chain of arachidonic acid in the conformations adopted by this compound when bound to various proteins. The coordinates of such complexes are available through the Protein Data Bank.21 Figure 6 shows the superposition of 9g on arachidonic acid in the conformation the latter adopts when interacting with human adiacyl lipid-binding protein,22 COX-1,23 and COX-2.24 It should be noted that the two phenyl rings of 9g can occupy regions of space corresponding to the first two cis double bonds of arachidonic acid. A folded conformation similar to that illustrated in Figure 5A was found, together with other more straight conformations, for arachidonic acid in complex with human serum albumin.25 Similar folded conformations also were observed for oleic acid and other unsaturated fatty acids bound to human fatty acid binding proteins (FABP).26 These observations led us to consider the m-biphenyl fragment as a putative bioisoster of the first moiety of such chains, being able to reproduce the bent shape of the first two cis-double bonds of the arachidonyl chain.

**Figure 4.** Lineweaver–Burke analysis of the hydrolysis of [3H]anandamide (1–100 µM) by rat brain membranes in the absence (■) or presence (□) of compound 7 (1 µM). Results are from one experiment, representative of five in which maximal velocities (V$_{\text{max}}$) for [3H]anandamide hydrolysis were 2271 ± 477 pmol/(min·mg) protein in control samples and 703 ± 188 pmol/(min·mg) protein in the presence of compound 7 (p < 0.05). Michaelis–Menten constants (K$_{M}$) were 9.3 ± 2.3 µM in control samples and 10.2 ± 3.3 µM in the presence of compound 7 (p > 0.05, Student’s t test; n = 5).

**Figure 5.** (A) Representation of minimum energy conformations of 22 (green carbons) and 7 (white carbons, in gauche and anti conformations) after superposition of the carbamate group. (B) Superposition of the biphenyl moiety of 9i (orange carbons) to the lipophilic chain of arachidonic acid, cocystalized with adipocyte lipid-binding protein (white carbons), COX-1 (yellow carbons), or COX-2 (green carbons).
The correlation between molecular shape and FAAH inhibition kinetics, these results support the notion that the two classes of compounds inhibit FAAH through separate mechanisms.

We next replaced the aromatic O-substituent with flexible alkyl chains. In the 2-aclyoxazolopyridine series of competitive FAAH inhibitors described by Boger and collaborators,11 the introduction of an O-phenylpentyl group leads to one of the most potent inhibitors. By contrast, in our series the presence of the same moiety was detrimental to the inhibitory activity (see Experimental Section). Together with their differences in inhibition kinetics, these results support the notion that the two classes of compounds inhibit FAAH through separate mechanisms.

A further indication of the distinct SAR profile of the N-alkylcarbamate aromatic esters described here is the fact that compound 11, which has a bent shape like 9g but inverted N- and O-substituents, was inactive. This suggests that the binding pockets of the two moieties have specific stereoelectronic requirements.

The superposition of the carbamate fragments and the phenyl rings (see Experimental Section) led to a PLS model with two latent variables endowed with appreciable descriptive and predictive power (r² = 0.82, s = 0.32, q²LOO = 0.54) for the 14-compound set, whose experimental and calculated pIC₅₀ values are reported in Table 2 and graphically plotted in Figure 6A. The coefficients of the steric field are represented in Figure 8 as isopotential surfaces. As can be observed from the green and blue surfaces corresponding to a positive and very positive correlation, respectively, between steric occupancy and potency, two favorable regions were detected by our model. The first (at the top right of Figure 6B) was due to the lower mean pIC₅₀ values for the N-butyl derivatives with respect to the N-cyclohexyl ones. It should be noted that this positive region would be much more relevant if the compounds having small N-substituents (19–21) could be included in the analysis, but this was precluded by the fact that only one of them had a measurable IC₅₀. As a consequence, the importance of the N-substituent is underestimated by our 3D-QSAR model, which, however, was developed essentially to investigate the O-substituent requirements. Indeed, a larger and deeper favorable region was observed for this moiety, as illustrated by the green and blue volumes at the bottom of Figure 6B, indicating the presence of steric bulk, positively correlated with inhibitory potency, around the meta position of the proximal ring of the O-biphenyl substituent. This region corresponds to the second ring of the naphthyl system (especially the areas about its 7 and 8 positions) and of the distal phenyl of the styryl substituent in its (Z)-configuration. It is reasonable to assume the proximity of this region to the binding site surface of FAAH, which would result in an improvement of dispersion forces and/or lipophilic interaction between

### Table 2. Experimental and Observed pIC₅₀ Values on FAAH Activity Inhibition for the N-Alkylcarbamate Aryl Esters Included in the 3D-QSAR Analysis

<table>
<thead>
<tr>
<th>compd</th>
<th>R₁</th>
<th>R₂</th>
<th>pIC₅₀</th>
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<th>calc</th>
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<tr>
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<td>6.22</td>
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<td>phenyl</td>
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<td>5.41</td>
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<tr>
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<td>5.94</td>
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<tr>
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<td>p-tolyl</td>
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<td>5.55</td>
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<tr>
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<tr>
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<tr>
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<tr>
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<td>3-pentylphenyl</td>
<td>6.73</td>
<td>6.83</td>
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Figure 6. (A) Plot of the experimentally observed pIC₅₀ data vs those calculated by the 3D-QSAR CoMSIA model for the 14 compounds reported in Table 2. A PLS model with two latent variables gave the following statistics: r² = 0.82, s = 0.32, q²LOO = 0.54. (B) 3D-QSAR graphical depiction of the coefficients for the CoMSIA fields, calculated by PLS analysis on the pIC₅₀ values of the 14 compounds reported in Table 2. All the compounds are represented as lines except 7 (white carbons) and 9g (orange carbons), which are represented as sticks. The colored volumes indicate the points where the influence of the steric potential on pIC₅₀ is more significant. The color codes are the following: blue, very positive; green, positive; yellow, negative (see Experimental Section).
the enzyme and the inhibitor. Thus, the O-aromatic moiety, which is hypothesized to serve as a leaving group in the reaction leading to enzyme carboxamoylation, would exert its effect on inhibitory potency at an early recognition stage of the process.

We observed a small region with moderately negative coefficients, represented by the yellow surface at the bottom left of Figure 6B, opposite the point of attachment of the phenyl ring to the carbamate group. It indicates that longer straight fragments can be accommodated at the binding pocket in a less efficient manner than the folded ones. The most relevant example is represented by p-biphenyl derivative 26, whose potency is much lower than that of its m-biphenyl isomer 9g.

We interpret the CoMSIA coefficients to indicate the existence of a large cavity with a curved shape in the active site of enzyme, where suitable O-substituents can be accommodated, so that the interaction of their carbonyl group with the active serine of the enzyme is facilitated. This result further highlights the similarity between the m-biphenyl moiety and the conformation of arachidonic acid bound to FABP (Figure 6), strengthening the initial hypothesis that FAAE bind to FAAH in a folded conformation, at least for the first 10 carbon atoms. We cannot exclude, however, that the fatty acyl chain of anandamide and the O-substituent of our carbamate esters are docked to different binding pockets within the FAAH active site. The availability of FAAH 3D coordinates, which were published for a covalent cation of the residue by column chromatography (cyclolhexane/ EtOAc 9:1 for 9a,b,h; 8:2 for 9i; 85:15 for 9c; 95:5 for 9g; CHCl3 for 9d,e) and recrystallization gave 9a-e-g-i.

Cyclohexylcarbamic Acid Phenyl Ester (9a). White needles. Yield: 73% (80 mg). Mp: 137 °C (MeOH) (lit. 128–30 °C from 50% EtOH).33 MS (EI): m/z 219 (M+), 94 (100). 1H NMR (CDCl3): δ 1.20–2.05 (m, 10H), 3.57 (m, 1H), 4.91 (br s, 1H), 7.11–7.40 (m, 5H) ppm. The IR spectrum is in agreement with literature.18 Anal. (C13H17NO2) C, H, N.

Cyclohexylcarbamic Acid m-Tolyl Ester (9b). White needles. Yield: 87% (130 mg). Mp: 117–118 °C (MeOH). MS (EI): m/z 233 (M+), 107 (100). 1H NMR (CDCl3): δ 1.18–2.05 (m, 10H), 2.35 (s, 3H), 3.57 (m, 1H), 4.90 (br s, 1H), 6.91–7.02 (m, 3H), 7.23 (t, 1H) ppm. IR (KBr): 3305, 1744, 1709 cm−1. Anal. (C13H17NO2) C, H, N.

Cyclohexylcarbamic Acid 8-Bromonaphthalen-2-yl Ester (9c). Yelllow needles. Yield: 66% (115 mg). Mp: 170–171 °C (MeOH). MS (EI): m/z 348 (M+), 114 (100). 1H NMR (CDCl3): δ 1.18–2.09 (m, 10H), 3.63 (m, 1H), 5.03 (br d, 1H), 7.26–7.99 (m, 6H) ppm. IR (KBr): 3316, 1737, 1708 cm−1. Anal. (C16H13BrNO2) C, H, N.

Cyclohexylcarbamic Acid 6-Ethynaphthalen-2-yl Ester (9d). White needles. Yield: 74% (110 mg). Mp: 162–164 °C (MeOH). MS (EI): m/z 297 (M+), 157 (100). 1H NMR (CDCl3): δ 1.17–2.07 (m, 13H), 2.81 (q, 2H), 3.61 (m, 1H), 4.97 (br d, 1H), 7.22–7.79 (m, 6H) ppm. IR (KBr): 3316, 1737, 1708 cm−1. Anal. (C14H17NO2) C, H, N.

(E)-Cyclohexylcarbamic Acid 4-Styrylphenyl Ester (9e). White crystals. Yield: 86% (138 mg). Mp: 165–166 °C (MeOH). MS (EI): m/z 321 (M+), 196 (100). 1H NMR (CDCl3): δ 1.18–2.05 (m, 10H), 3.56 (m, 1H), 4.75 (br d, 1H), 7.03 (d, 1H, J = 16.7), 7.12 (d, 1H), 7.13 (d, 2H), 7.26–7.52 (m, 7H) ppm. IR (KBr): 3440, 1736, 963 cm−1. Anal. (C20H17NO2) C, H, N.

Cyclohexylcarbamic Acid Biphenyl-3-yl Ester (9f). White crystals. Yield: 97% (143 mg). Mp: 141–143 °C (MeOH). MS (EI): m/z 270 (100). 1H NMR (CDCl3): δ 1.22–2.06 (m, 10H), 3.59 (m, 1H), 4.94 (br d, 1H), 7.12–7.61 (m, 9H) ppm. IR (KBr): 3440, 1733 cm−1. Anal. (C20H18NO2) C, H, N.

Cyclohexylcarbamic Acid 3-Pentylphenyl Ester (9h). White crystals. Yield: 98% (142 mg). Mp: 94–95 °C (petroleum ether). MS (EI): m/z 259 (M+), 164 (100). 1H NMR (CDCl3): δ 0.89 (t, 3H), 1.18–2.05 (m, 16H), 2.60 (t, 2H), 3.57 (m, 1H), 4.88 (br d, 1H), 6.93–7.27 (m, 4H) ppm. IR (KBr): 3306, 1743, 1705 cm−1. Anal. (C19H22NO2) C, H, N.

Cyclohexylcarbamic Acid 5-Phenylpentyl Ester (9i). White needles. Yield: 80% (115 mg). Mp: 55–56 °C (EtO/ petroleum ether). MS (EI): m/z 289 (M+), 146 (100). 1H NMR (CDCl3): δ 0.96–1.03 (m, 1H), 1.06–1.96 (m, 16H), 2.62 (t, 2H), 3.49 (m, 1H), 4.04 (d, 1H, J = 17.1), 4.53 (br s, 1H), 7.16–7.28 (m, 5H) ppm. IR (KBr): 3442, 1703 cm−1. Anal. (C19H23NO2) C, H, N.

Synthesis of (Z)-Cyclohexylcarbamic Acid 4-Styrylphenyl Ester (9j). A stirred mixture of benzyltriphenylphosphonium chloride (16.8 g, 43.3 mmol), 4-hydroxybenzaldehyde (5.28 g, 43.3 mmol), DBU (6.8 g, 45 mmol), and CH2CN (67 mL) was refluxed for 4 h and concentrated. Purification of the residue by column chromatography (cyclohexane/ EtOAc 9:1 for 9a,b,h; 8:2 for 9i; 85:15 for 9c; 95:5 for 9g; CHCl3 for 9d,e) and recrystallization gave 9a-e-g-i.
residue by column chromatography (cyclohexane/EtOAc 85:15) gave pure E-stilben-4-ol (2 g) and a 1:1 (NMR) mixture of Z- and E-stilben-4-ols (0.456 g, 2.33 mmol). This was dissolved in toluene (13 mL), and Et3N (0.014 g, 0.019 mL, 0.14 mmol) and c-C6H5NCO (0.32 g, 0.33 mL, 2.57 mmol) were added. The mixture was refluxed under stirring for 3 h, cooled, and concentrated. Purification of the residue by column chromatography (cyclohexane/EtOAc 85:15) gave the desired product along with a small amount of confirmation. Recrystallization gave 16f as a white solid. Mp: 131–133 °C (EtOH/H2O). MS (EI): m/z 321 (M+) 190 (100). 1H NMR (CDCl3): δ 11.9–2.04 (m, 10H), 3.54 (m, 1H), 4.88 (br d, 1H), 6.54 (d, 1H, J = 12.2), 6.60 (d, 1H, J = 12.2), 6.98 (d, 2H), 7.24 (m, 7H) ppm. IR (KBr): 3404, 1730 cm–1. Anal. (C17H19NOS) C, H, N.

Synthesis of Biphenyl-3-yl Carbamic Acid Cyclohexyl Ester (11). To a stirred solution of biphenyl-3-ylamine (90 mg, 0.53 mmol) in CH2Cl2 (2 mL), diimidazol-1-ylmethane (10) (345 mg, 2.13 mmol) and DMAP (12 mg, 0.1 mmol) were added. The mixture was reacted at room temperature for 2 h, cool and concentrated. Purification of the residue by column chromatography (cyclohexane/EtOAc 9:1) and recrystallization gave 11 as a white solid. Yield: 55% (86 mg). Mp: 127–129 °C (EtOH). MS (EI): m/z 295 (M+), 141 (100). 1H NMR (CDCl3): δ 1.30–2.03 (m, 10H), 4.78 (m, 1H), 6.66 (br s, 1H), 7.31–7.47 (m, 6H), 7.59–7.70 (m, 3H) ppm. IR (KBr): 3350, 1725, 1689 cm–1. Anal. (C17H17NO3) C, H, N.

Synthesis of Biphenyl-3-yl Carbamic Acid Cyclohexyl Ester (16a). A stirred mixture of biphenyl-3-ylamine (15a) (143 mg, 1 mmol), c-C6H5NH2 (99 mg, 0.11 mL, 1.1 mmol), and Et3N (5 mg, 0.07 mL, 0.05 mmol), and toluene (5 mL) was refluxed for 20 h. Two further amounts of 16a (69 mg, 0.07 mL, 0.55 mmol) were added during this period, after 5 and 3 h, respectively. The mixture was then cooled and concentrated. Purification of the residue by column chromatography (cyclohexane/EtOAc 7:3) and recrystallization gave 16a as white solid. Yield: 60% (86 mg). Mp: 154–155 °C (EtOH). MS (EI): m/z 295 (M+), 141, 115 (100). 1H NMR (CDCl3): δ 1.22–2.22 (m, 10H), 4.14 (m, 1H), 6.64 and 6.84 (1H), 7.24–7.32 (m, 1H), 7.46–7.54 (m, 3H), 7.79–7.90 (m, 3H) ppm. IR (Nujol): 3212, 1623 cm–1. Anal. (C17H17NO2) C, H, N.

(b) Pharmacology. Cell fractions were prepared from brain homogenates, and membrane FAAH activity was assayed using anandamide (ethanamide-H1) (American Radiolabeled Chemicals, ARC (St. Louis, Missouri), 60 Ci/mmol) as a substrate. 1H Anandamide transport assays were conducted in human astrocytoma cells, preincubating cells with inhibitors for 10 min at 37 °C, prior to exposure to [3H]anandamide for 4 min. CB1 and CB2 binding assays were conducted in rat cerebellar membranes (27000g) and CB2-overexpressing CHO cells (purchased from Receptor Biology-Perkin Elmer, Welleley, MA), respectively, using [3H]WIN-55212-2 (NEN-Dupont, Boston, MA, 40–60 Ci/mmol, 10 nM) as a ligand. Cholinesterase assays were conducted with a commercial kit (Sigma, St. Louis, MO), using pig liver enzymes (electric eel acetylcholinesterase type V-S and horse-serum cholinesterase, both from Sigma, St. Louis, MO) and following vendor’s instructions.

(c) Molecular Modeling. Molecular models were built by applying the standard tools in Sybyl, version 6.6 (Tripos Inc., 1699 South Hanley Rd, Louis, MO, 63144). Their geometries were optimized by energy minimization, employing the Merck molecular force field 94s (MFF94s), implemented in Sybyl. Conformational analysis was performed by systematic scanning of the rotatable bonds and energy minimization to a gradient of 0.01 kcal/mol. The conformers with the highest intersection volume with the template structure were employed for compound alignment in the following analysis. For 3D-QSAR analysis, minimum energy conformations of the compounds were aligned by a rigid root-mean-square (rms) fit of the five atoms in the carbamate fragment and the six carbon atoms in the closer phenyl ring, employing 9g as a template structure. For compounds 7 and 9f, the centroid of the distal phenyl ring was also superposed to that of 9g. The CoMSIA27 models were used for the quantitative activity study. The CoMSIA steric field was calculated with a lattice with a grid resolution of 2 Å, whose extension was at least 4 Å beyond every molecular boundary in all directions. An sp3 carbon was taken as the probe atom. Regression analyses were performed using the PLS58 algorithm in Sybyl. The cross-validation technique with the leave-one-out (LOO)
procedure\textsuperscript{66} was applied to calculate \(q_1^2\), as a rough estimate of the predictive power and to choose the optimal number of latent variables. Variables with an energy standard deviation lower than 2 kcal/mol were discarded to minimize the influence of noisy couplings and to speed the computation. The final non-cross-validated analyses were derived with the number of latent variables corresponding to the first maximum cross-validated analyses were derived with the number of latent variables.

The contour volumes of the 3D-QSAR model, represented in Figure 6B, correspond to the following cutoff values for the product of the PLs coefficients and the standard deviation of the steric potential: (blue) \( \geq 0.05 \), (green) \( > 0.05 \), (yellow) \( > 0.01 \), (red) \( > 0.05 \).

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References


(8) Acknowledged for supplying the Sybyl software license.
(34) The signals at 7.02 (d, J = 6.3) and 6.84 (d, J = 6.3) ppm were attributed to (E)-stilben-4-ol. Those at 7.14 (d, J = 8.8) and 6.69 (d, J = 8.8) ppm were attributed to the (Z)-isomer, and the rest of the spectrum is consistent with the proposed structure.
(37) The NH proton appears to be split into two broad doublets, possibly because of tautomeric forms involving the C(S)=N bond.