Cyclohexylcarbamic Acid 3′- or 4′-Substituted Biphenyl-3-yl Esters as Fatty Acid Amide Hydrolase Inhibitors: Synthesis, Quantitative Structure−Activity Relationships, and Molecular Modeling Studies

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Fatty acid amide hydrolase (FAAH) is a promising target for modulating endocannabinoid and fatty acid ethanolamide signaling, which may have important therapeutic potential. We recently described a new class of *O*-arylcarbamate inhibitors of FAAH, including the cyclohexylcarbamic acid biphenyl-3-yl ester URB524 (half-maximal inhibitory concentration, $IC_{50} = 63$ nM), which have significant anxiolytic-like properties in rats. In the present study, by introducing a selected group of substituents at the meta and para positions of the distal phenyl ring of URB524, we have characterized structure−activity profiles for this series of compounds and shown that introduction of small polar groups in the meta position greatly improves inhibitory potency. Most potent in the series was the *m*-carbamoyl derivative URB597 (**4i**, $IC_{50} = 4.6$ nM). Furthermore, quantitative structure−activity relationship (QSAR) analysis of an extended set of meta-substituted derivatives revealed a negative correlation between potency and lipophilicity and suggested that small-sized substituents may undertake polar interactions with the binding pocket of the enzyme. Docking studies and molecular dynamics simulations, using the crystal structure of FAAH, indicated that the *O*-biphenyl scaffold of the carbamate inhibitors can be accommodated within a lipophilic region of the substrate-binding site, where their folded shape mimics the initial 10−12 carbon atoms of the arachidonyl moiety of anandamide (a natural FAAH substrate) and methyl arachidonyl fluorophosphonate (a nonselective FAAH inhibitor). Moreover, substituents at the meta position of the distal phenyl ring can form hydrogen bonds with atoms located on the polar section of a narrow channel pointing toward the membrane-associated side of the enzyme. The structure−activity characterization reported here should help optimize the pharmacodynamic and pharmacokinetic properties of this class of compounds.

Introduction

Fatty acid amide hydrolases (FAAHs)[1,2] are intracellular enzymes responsible for the hydrolysis of endogenous fatty acid ethanolamides (FAEs),[3,4] a reaction that, along with transport into cells,[5−7] terminates the biological effects of these lipid mediators. At least two distinct classes of cellular receptors are thought to mediate such effects. The polyunsaturated FAE anandamide[8] (arachidonoylethanolamide, **1**, Figure 1) activates cannabinoid receptors, G protein-coupled receptors found in brain and immune cells that may play essential roles in the intrinsic regulation of pain, anxiety, and memory.[1] On the other hand, the monounsaturated FAE oleoylethanolamide[9,10] (OEA, 18:1Δ^9) activates the peroxisome proliferator-activated receptor-α (PPAR-α), a nuclear receptor involved in the control of satiety, energy metabolism, and inflammation. The saturated FAE palmitoylethanolamide[11,12] (PEA, 16:0) also is biologically active, but its mechanism of action is still undefined.

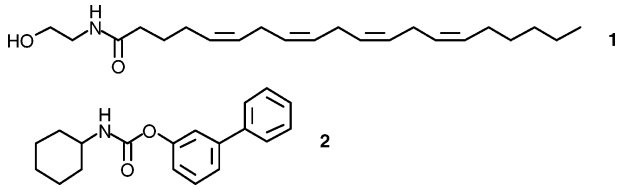

Figure 1. Chemical structures of anandamide (**1**) and URB524 (**2**).

Owing to the emerging physiological functions of the FAEs, small-molecule inhibitors that selectively target intracellular FAAH activity may not only be useful as research tools but also serve as prototypes for the development of novel therapeutic agents.[13−18] Therefore, starting from the assumption that carbamic acid esters may act as site-directed inhibitors of FAAH, we have developed a series of *O*-aryl-*N*-alkylcarbamic acid esters, which are highly potent at inhibiting FAAH activity both in vitro and in vivo, but do not significantly interact with several other serine hydrolases (e.g. acetylcholinesterase and monoglyceride lipase) or with cannabinoid receptors. In rats, these compounds display profound anxiolytic-like properties, which may be attributed to their ability to elevate brain anandamide levels.[19]

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An initial structure−activity relationship (SAR) investigation into the steric requirements for the lipophilic *O*-aryl moiety of these compounds suggested that nonlinearly shaped structures may be associated with greater inhibitory potencies.[20] This idea is supported by two findings. First, the curved shape of the most successful FAAH inhibitors resembles the folded conformation assumed by fatty acids in complex with binding proteins, as well as one of the conformations predicted for anandamide binding to the CB_1 cannabinoid receptor.[21,22] Second, and more importantly, the crystal structure of FAAH inhibited by methyl arachidonyl fluorophosphonate (MAPF) revealed that the arachidonyl chain of this covalent inhibitor assumes a folded conformation in the substrate-binding site of the enzyme.[23]

Previous three-dimensional quantitative SAR (3D-QSAR) analyses of the alkylcarbamic acid aryl esters[20] indicated that space occupancy of a region corresponding to the meta position of an *O*-phenyl ring is positively correlated with FAAH inhibition, which is in turn suggestive of a favorable interaction of the inhibitor with the enzyme binding site. The most potent compound in this series was the biphenyl-3-yl derivative URB524 (**2**, Figure 1), which inhibited FAAH activity in rat brain membranes with a half-maximal concentration (IC_{50}) value of 63 nM. These experiments did not attempt, however, to identify the lipophilic and electronic, but only the steric, requirements of the binding site. Therefore, in the present study we have taken URB524 as the starting point for a systematic exploration of the effect of phenyl substitution on FAAH inhibition, introducing at the meta and para positions of the distal phenyl ring of URB524 a set of substituents with balanced variation in their lipophilic and electronic properties. The distal ring has been selected for two reasons. First, our previous 3D-QSAR analyses suggested that this moiety is critical to achieve significant inhibitory potency. Second, substitutions on the distal ring are in principle devoid of direct resonance effects upon the carbamate group, and are expected therefore to yield compounds the potencies of which should correlate directly with noncovalent binding interactions.

Our experimental design comprised two steps. We first tested the sensitivity of the meta and para positions of the distal phenyl ring to the lipophilic and electronic properties of a small set of moderate-size substituents. Next, after the more responsive position of the phenyl ring was identified, we expanded the series of substituents while maintaining significant and independent variation among the variables describing their lipophilic, electronic, and steric properties, as required to investigate QSARs by multiple regression analysis (MRA).[24,25]

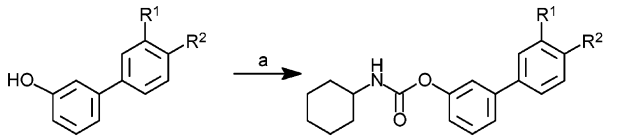
Chemistry

Cyclohexylcarbamic acid aryl esters **4a**−**c**,**e**−**i**,**l**,**k**,**m**−**w** and **4x**,**y** were obtained by addition of cyclohexylisocyanate to phenylphenols **3a**−**c**,**e**,**g**−**i**,**k**,**m**−**w** (Scheme 1), or **3x**,**y** (Scheme 3), respectively. Removal of the protective benzyloxycarbonyl from **4x**,**y** afforded **4j**,**d** (Scheme 3). **4z** was prepared by hydrogenation of **4t**.

Compounds **3a**−**e**,**g**,**h**,**j**,**k**,**m**−**o**,**q** were obtained by 57% hydriodic acid (**3a**,**b**,**d**,**e**,**g**,**h**,**j**,**k**,**m**,**n**), or boron tribromide (**3c**,**o**,**q**), hydrolysis of opportune 3-methoxybenzenes. The latter compounds were prepared by Suzuki coupling of arylboronic acid **5a** and a halobenzene (**6a**−**e**,**g**,**h**,**j**,**k**,**m**,[26]**n**,**o**,[27]**q**) (Scheme 2). **6i** was prepared from 3-bromobenzonitrile, employing sodium perborate.[28] Compounds **3i**,**r**−**w** were directly synthesized from the commercially available 3-hydroxyphenylboronic acid (**5b**) (Scheme 2).

The synthesis of **3p** is reported in Scheme 4: the propenyl derivative **11**, obtained by a Wittig reaction from aldehyde **9** was reduced to give **8p**, which was eventually hydrolyzed to furnish the desired biphenol.

Scheme 1[a]

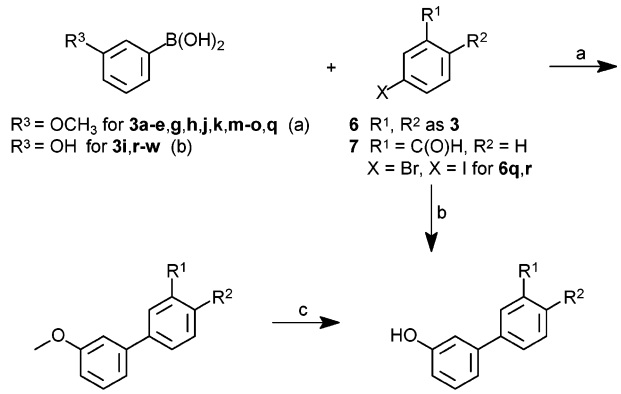


3a R^1 = H R^2 = CF_3
3b R^1 = H R^2 = CH_3
3c R^1 = H R^2 = $C(O)NH_2$
3e R^1 = H R^2 = F
3g R^1 = CF_3 R^2 = H
3h R^1 = CH_3 R^2 = H
3i R^1 = $C(O)NH_2$ R^2 = H
3k R^1 = F R^2 = H
3m R^1 = C_6H_5O R^2 = H
3n R^1 = C_6H_5 R^2 = H
3o R^1 = $CH_2C_6H_5$ R^2 = H
3p R^1 = *n*-C_3H_7 R^2 = H
3q R^1 = NO_2 R^2 = H
3r R^1 = SO_2NH_2 R^2 = H
3s R^1 = $C(O)CH_3$ R^2 = H
3t R^1 = CN R^2 = H
3u R^1 = OH R^2 = H
3v R^1 = CH_2OH R^2 = H
3w R^1 = $(CH_2)_2OH$ R^2 = H

4a-c,**e**,**g-i**,**k**,**m-w** as **3a-c**,**e**,**g-i**,**k**,**m-w**
4f R^1 = H R^2 = C(O)NHC(O)NH*c*-C_6H_{11}
4l R^1 = OC(O)NH*c*-C_6H_{11} R^2 = H

[a] Reagents and conditions: (a) c-$C_6H_{11}NCO$, Et_3N, toluene, reflux, 1−32 h.

Scheme 2[a]



5a R^3 = OCH_3 for **3a-e**,**g**,**h**,**j**,**k**,**m-o**,**q** (a)
5b R^3 = OH for **3i**,**r-w** (b)

6 R^1, R^2 as **3**
7 R^1 = C(O)H, R^2 = H
X = Br, X = I for **6q**,**r**

8a-c,**e**,**g**,**h**,**k**,**m-o**,**q** as in scheme 1
8j R^1 = NH_2, R^2 = H
8d R^1 = H, R^2 = NH_2
9 as **7**

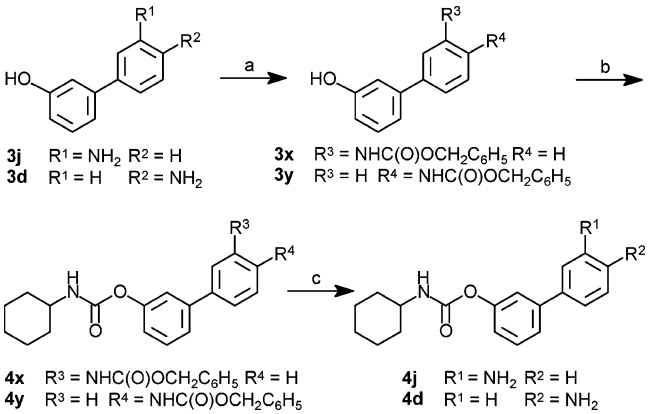
3a-c,**e**,**g-i**,**k**,**m-w** as in scheme 1
3j R^1 = NH_2, R^2 = H
3d R^1 = H, R^2 = NH_2

[a] Reagents and conditions: (a) $Pd(PPh_3)_4$, toluene, Na_2CO_3/H_2O, EtOH, reflux, 1−3 h; (b) reaction conditions as in (a); (c) HI, reflux, 2−7.5 h for **3a**,**b**,**d**,**e**,**g**,**h**,**j**,**k**,**m**,**n** or BBr_3, 25 °C, 1−18 h for **3c**,**o**,**q**.

Results and Discussion

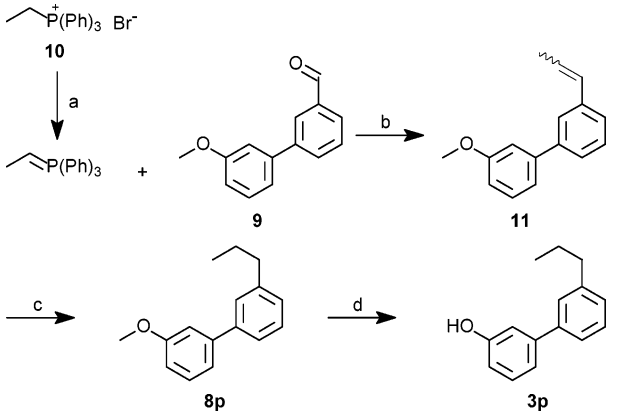
We measured FAAH activity in rat brain membranes, using [^{3}H]anandamide as a substrate. The IC_{50} values for compounds **4a**−**z** are reported in Table 1. At 30−100 μM, the compounds (i) had no effect on acetylcholinesterase activity (electric eel) or butyrylcholinesterase

Scheme 3[a]



[a] Reagents and conditions: (a) BzOC(O)Cl, $NaHCO_3/H_2O$, 20 h; (b) c-C_6H_{11}NCO, Et_3N, toluene, reflux, 8−32 h; (c) H_2, 10% Pd/C, 3 atm, THF, MeOH, 65 °C, 24 h.

Scheme 4[a]

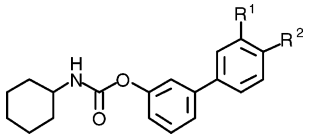


[a] Reagents and conditions: (a) 2 M *n*-BuLi in hexane, N_2, 0 °C, 2 h; (b) −78 to 25 °C, reflux, 20 h; (c) H_2, PdO_2, 3 atm, 3 h; (d) HI, reflux, 3 h.

activity (horse serum); (ii) did not displace the binding of [^{3}H]WIN-55212-2 (10 nM) to rat CB_1 cannabinoid receptors or human recombinant CB_2 receptors; and (iii) did not affect [^{3}H]anandamide (100 nM) transport in human astrocytoma cells in culture (data not shown).

We first examined a small set of URB524 analogues (**4a**−**l**) the substituents of which (methyl, trifluoromethyl, amino, and carbamoyl) represent the four combinations of positive and negative levels for the π and σ (σ_m or σ_p) descriptors; small (fluoro) and bulky (cyclohexylcarbamoyloxy or cyclohexylureidocarbonyl) substituents were also included in this explorative set. The results suggested that substitutions in the meta position of the distal phenyl ring yield the most potent inhibitors. Thus, the 3′-methyl (**4h**) and 3′-amino (**4j**) derivatives were as potent as the parent compound (URB524), while the 3′-carbamoyl derivative **4i** (URB597) was an order of magnitude more potent than URB524. By contrast, with the only exception of the 4′-fluoro derivative **4e**, all para-substituted compounds were less active than URB524. In contrast with the general SAR trend, according to which meta-substituted compounds were more active than the corresponding para isomers, 3′-fluoro derivative **4k**, the meta isomer of **4e**, was less active than URB524. The requirements of the group matching the area of the receptor near the 4′-position seem more strict than those of the 3′-group, as hinted by the comparison

Table 1. Inhibitory Potencies (IC_{50}) of Tested Compounds on FAAH Activity



compd	R^1	R^2	IC_{50} (nM) ± SEM
URB524	H	H	63 ± 9
4a	H	CF_3	1587 ± 148
4b	H	CH_3	155.4 ± 31.0
4c	H	$C(O)NH_2$	5909 ± 951
4d	H	NH_2	360 ± 59
4e	H	F	64.95 ± 14.00
4f	H	C(O)NHC(O)NH-c-C_6H_{11}	3017 ± 688
4g	CF_3	H	145.7 ± 16.0
4h	CH_3	H	61.75 ± 19.00
4i	$C(O)NH_2$	H	4.6 ± 1.6
4j	NH_2	H	64.6 ± 9.0
4k	F	H	96.6 ± 4.0
4l	OC(O)NH-c-C_6H_{11}	H	361 ± 137
4m	C_6H_5O	H	420 ± 86
4n	C_6H_5	H	565 ± 42
4o	$CH_2C_6H_5$	H	1857 ± 57
4p	*n*-C_3H_7	H	110 ± 16
4q	NO_2	H	49.6 ± 2.0
4r	SO_2NH_2	H	26.5 ± 4.5
4s	$C(O)CH_3$	H	9.1 ± 0.5
4t	CN	H	33.9 ± 7.0
4u	OH	H	8.65 ± 0.10
4v	CH_2OH	H	8.67 ± 0.90
4w	$(CH_2)_2OH$	H	18.7 ± 4.5
4z	CH_2NH_2	H	21177 ± 7277

of IC_{50} ratio of compounds **4b**,**4a** and **4h**,**4g**, respectively. Given the results of this first set of derivatives, further modifications at the 4′-position were discharged. Moreover, compounds **4f** and **4l** demonstrated that the introduction of more extended fragments is detrimental to activity.

In order to search for statistical relationships between physicochemical properties and inhibitor potency, we inserted at the meta position twelve additional substituents (**4m**−**z**, Table 1), which adequately span the space of lipophilic, steric, and electronic properties. Certain substituents were also selected for their topological similarity with the carbamoyl group (**4r**) or with portions of it (**4s**, **4v**, or **4z**). The IC_{50} values for **4m**−**z** indicate that hydrophilic (**4q**−**w**) groups have a favorable impact on pharmacological activity. On the contrary, the introduction of large lipophilic groups (**4m**−**p**) is detrimental to activity. Several compounds in this set are more active than URB524, although none of them is better than the *m*-carbamoyl derivative URB597 (**4i**).

The nineteen (including hydrogen) biphenyl substituents listed in Table 1 constitute a set with a large variation in both lipophilicity (almost 4 π units) and steric bulk (35 MR units). Furthermore, π and molar refractivity (MR) values are practically uncorrelated to the electronic effects (r with σ_m of −0.19 and −0.16, respectively), though some correlation is present between lipophilic (π) and steric (MR) descriptors (r = 0.63), due to the known difficulty in obtaining big hydrophilic substituents.

Multiple regression analysis (MRA) employing eight common physicochemical descriptors (π, σ_m, F, R, MR,

Table 2. QSAR Analysis: Correlation Matrix for the Nineteen Meta Substituents of Compounds URB524 and **4g**−**z**

	MR	π	σ_m	σ_p	F	R	L	B_1
π	0.63							
σ_m	−0.16	−0.19						
σ_p	−0.02	−0.14	0.89					
F	−0.19	−0.15	0.87	0.60				
R	0.05	−0.12	0.60	0.89	0.19			
L	0.86	0.42	−0.06	0.13	−0.15	0.23		
B_1	0.12	−0.07	0.49	0.59	0.29	0.56	0.30	
B_5	0.94	0.52	−0.14	−0.02	−0.18	0.06	0.75	0.11

L, B_1, B_5, see the correlation matrix in Table 2) and π^2 did not yield a statistically significant model. However, a simple plot of pIC_{50} vs lipophilicity (not shown) indicated a clear relationship, albeit masked by the presence of an outlier, the aminomethyl derivative **4z**. This can be attributed to the basicity of the compound, which is expected to be largely protonated at neutral pH. When **4z** was omitted from the regression set, the relationship between lipophilicity and potency gave the regression equation 1.

$$pIC_{50} = -0.49(\pm 0.07)\pi + 7.26(\pm 0.09) \qquad (1)$$

$$n = 18 \text{ (URB524, } \mathbf{4g}-\mathbf{w}) \qquad r^2 = 0.74 \qquad s = 0.37$$

$$F = 46.0 \qquad q^2 = 0.66 \qquad \text{SDEP} = 0.40$$

The negative correlation with lipophilicity was unexpected in that we had previously hypothesized that the biphenyl moiety may mimic the fatty acyl chain of a FAE bound to the hydrophobic binding site of FAAH. Yet, no MRA model including up to five variables turned out to be statistically better than, or equivalent to, that represented in eq 1. Only the inclusion of an indicator variable, set to one for substituents able to give hydrogen bonds (HB), and to zero in other cases, allowed the detection of an alternative model (eq 2) having comparable descriptive (r^2) and predictive (q^2) power. The pIC_{50} values calculated by eq 2 are reported in Table 3, together with independent variables employed in the QSAR models.

$$pIC_{50} = -0.046(\pm 0.009)\,\text{MR} + 0.80(\pm 0.18)\,\text{HB} + 7.29(\pm 0.17)$$

$$n = 18 \text{ (URB524, } \mathbf{4g}-\mathbf{w}) \qquad r^2 = 0.76 \quad s = 0.37$$

$$F = 23.2 \quad q^2 = 0.68 \quad \text{SDEP} = 0.39 \qquad (2)$$

Although this model is more complex than that represented by eq 1, it could provide a possible interpretation for the positive effect of substituent hydrophilicity within a supposedly lipophilic binding pocket: eq 2 relates this behavior to the formation of hydrogen bonds between meta substituents and polar amino acid residues of the enzyme. This kind of interaction is strictly dependent on distances and angles between the atoms involved, which may explain the fact that, in the case of para-substituted compounds, no positive effect for the polar carbamoyl (**4c**) and amino (**4d**) substituents was observed. The last compounds of the series (**4r**−**w**), prepared to validate the temporary QSAR models built with the data previously available, confirmed the indications reported above, even if none of them resulted more potent than the carbamoyl derivative **4i**.

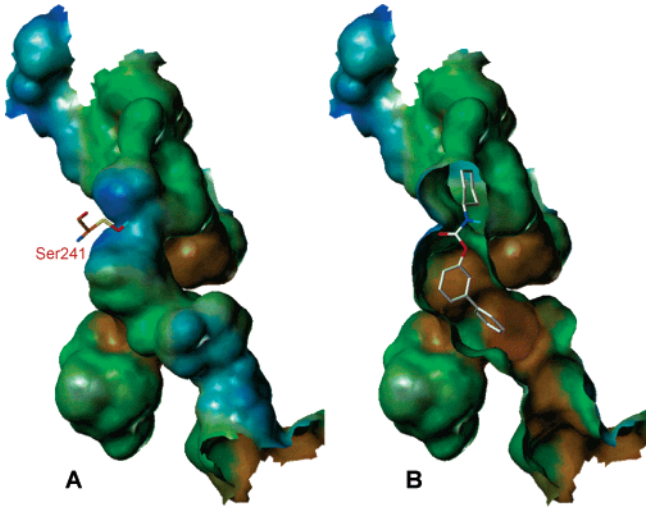
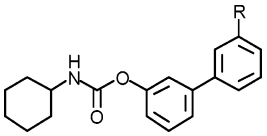


Figure 2. Surface of the catalytic channel inside FAAH, colored by a lipophilicity scale going from high lipophilicity (brown) to high hydrophilicity (blue). The channel has an outlet pointing to the membrane (bottom) and a funnel with a large opening toward the cytosol (high portion of figure, back). Left: whole surface of the channel with catalytic Ser241 displayed. Right: vertical section of the channel (the frontal has been erased, so as to display the docked structure of URB524).

Table 3. QSAR Analysis: Substituent Variables Employed in Eqs 1 and 2 and Observed pIC_{50} Values for Meta-Substituted Derivatives of **2** and Those Calculated by Eq 2



					pIC_{50}	
compd	R	π	MR	HB	obsd	calcd
URB524	H	0.00	1.03	0	7.20	7.24
4g	CF_3	0.88	5.02	0	6.84	7.06
4h	CH_3	0.56	5.65	0	7.21	7.03
4i	$C(O)NH_2$	−1.49	9.81	1	8.34	7.64
4j	NH_2	−1.23	5.42	1	7.19	7.84
4k	F	0.14	0.92	0	7.02	7.25
4l	$OC(O)NH\text{-}c\text{-}C_6H_{11}$	1.06	36.13	1	6.44	6.43
4m	C_6H_5O	2.08	27.68	1	6.38	6.82
4n	C_6H_5	1.96	25.36	0	6.25	6.12
4o	$CH_2C_6H_5$	2.01	30.01	0	5.73	5.91
4p	$n\text{-}C_3H_7$	1.55	14.96	0	6.96	6.60
4q	NO_2	−0.28	7.36	1	7.30	7.75
4r	SO_2NH_2	−1.82	12.28	1	7.58	7.53
4s	$C(O)CH_3$	−0.55	11.18	1	8.04	7.58
4t	CN	−0.57	6.33	1	7.47	7.80
4u	OH	−0.67	2.85	1	8.06	7.96
4v	CH_2OH	−1.03	7.19	1	8.06	7.76
4w	$(CH_2)_2OH$	−0.77	11.8	1	7.73	7.55

The resolution of the crystal structure of FAAH covalently bound to methyl arachidonyl phosphonate (MAP)[23] allowed us to rationalize our QSAR models by performing docking, and molecular dynamics (MD) simulations, experiments. Bracey et al. observed that the binding site for MAP lies in a channel that spans the entire length of the enzyme, from the membrane-bound surface (Figure 2A, bottom) to the cytosol at the opposite side (Figure 2A, top). The surface of this channel, colored according to lipophilicity, is represented

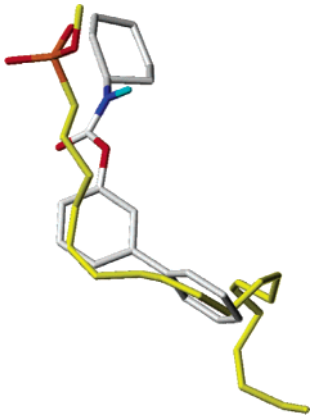


Figure 3. Superposition of the biphenyl fragment of URB524 (white carbons) to the first two double bonds of the MAP arachidonyl chain (yellow carbons), in the conformation observed in the crystallized complex with FAAH.[23]

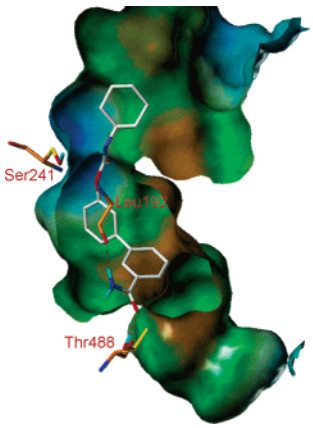


Figure 4. Docking of URB597 into the FAAH binding site. Carbons of the inhibitor are colored in white, those of FAAH side chains in yellow, and those of the FAAH backbone in orange; other atoms are colored following the usual atom code. The hydrogen bonds of the carbamoyl group of URB597 with the enzyme are evidenced. The surface of the enzyme channel has been left-rotated approximately 90° with respect to that represented in Figure 2.

in Figure 2. The channel has a complex topography: it comprises a central hydrophilic area around the catalytic residue Ser241, which is surrounded in turn by extended lipophilic surfaces. Proceeding toward the membrane, the duct bifurcates into a lipophilic bulge (lower part of Figure 2A, back), in which the terminal atoms of the arachidonyl chain of MAP are located, and a narrow tunnel with a hydrophilic ridge (lower part of Figure 2A, front). The polar head of the FAEs might temporarily link to this ridge en route to the catalytic site.

After deletion of the MAP fragment from the enzyme and refinement of the protein model (see Experimental Section), we performed molecular docking with URB524 and URB597 (**4i**). The results of these analyses showed that the biphenyl moiety of URB524 may readily replace the arachidonyl chain of MAP, with the meta position of the distal phenyl ring pointing exactly toward the hydrophilic ridge (Figure 2B). Indeed, a superposition placing the first two double bonds of the arachidonyl chain of MAP, in the conformation of its complex with FAAH, onto the biphenyl moiety of URB524 highlights the steric similarity of the two fragments and supports our initial hypothesis (Figure 3).

Docking of URB597, the most active compound of the present series, suggests that its carbamoyl group may form two distinct hydrogen bonds: one as an acceptor, with the hydroxyl group of Thr488, and the other as a donor, with the main chain carbonyl group of Leu192 (Figure 4). Docking of other meta-substituted structures, with the common biphenyl scaffold held in the same lipophilic region as in Figure 4, showed that all polar groups can form hydrogen bonds with the enzyme. However, the hydrogen bond of the carbamoyl group of URB597 is the strongest, possibly because its bidentate structure is absent in the acetyl and hydroxymethyl derivatives **4s** and **4v**. Accordingly, URB597 is more potent than the latter compounds (Table 1). This structure-based analysis, evidencing a role for specific hydrogen bonding interactions of our inhibitors with FAAH, may account for the empirical correlation described by eq 2 and provide an explanation for the negative correlation with lipophilicity (eq 1). The low tolerance to para substitution could be explained by the observation that, in the docking mode described above, the para position resulted so close to the side chains of Phe381, Phe432, and Leu404 that only a hydrogen or fluorine atom may avoid clashes with their aryl or alkyl groups (data not shown).

The fact that URB597 is the most potent inhibitor of our series prompted us to investigate in greater detail its interaction with FAAH using theoretical methods. Docking analysis revealed that a binding mode alternative to the one described above might be possible for this compound. In fact, a 180° rotation of the molecule (i) brings the *N*-cyclohexyl ring in a portion of the space occupied by the arachidonyl chain of MAP; and (ii) allows the biphenyl scaffold to lie in the funnel pointing to the cytosolic outlet, which is part of the FAAH channel. In this orientation it is still possible for the carbamoyl group of URB597 to form two hydrogen bonds, one with the backbone carbonyl of Leu380 and the other with the side-chain NH_2 of Gln273 (data not shown).

To test which of the two orientations is more stable we performed MD runs, maintaining the tertiary structure of FAAH observed in its crystallized complex with MAP and allowing movement of amino acid residues located at a maximum distance of 8 Å from the docked carbamate inhibitor. Starting from the orientation of Figure 4, URB597 gave a stable oscillation around a conformation very similar to its initial one, with an average distance between the nucleophilic oxygen of Ser241 and the electrophilic carbamate carbon of 3.00 ± 0.15 Å (mean ± SD). On the other hand, when MD runs were started from the alternative orientation, the ligand position was much less stable, the average distance between the two centers being of 4.10 ± 0.65 Å. Moreover, the *m*-carbamoyl group of URB597 left its original position and was able to undergo alternative polar interactions only at the cost of a worse adaptation of the carbamate fragment to the catalytic site. These results suggest that the binding mode depicted in Figure 4 may favor the nucleophilic attack of Ser241 after substrate recognition.

The stabilizing effect of the 3′-carbamoyl group in this binding orientation was further confirmed by comparing the MD trajectory of URB597 with that of its parent

compound URB524. The first structure showed reduced fluctuations, with respect to the initial conformation, indicating that the additional interaction provided by the carbamoyl group contributes to limit the movements of the inhibitor within the active site; this increases the probability of a reaction between Ser241 and the carbamate group, and could be related to the observed improvement of potency conferred by polar substituents in the 3′-position.

In conclusion, our findings indicate that the biphenyl scaffold in *O*-arylcarbamate inhibitors of FAAH may mimic the initial fatty acyl segment of anandamide and other FAEs, as they interact with the enzyme's active site. The results further suggest that polar substituents in the meta position of the distal phenyl group can form hydrogen bonds with hydrophilic amino acid residues within the FAAH channel. MD simulations supported this hypothesis, showing that such hydrogen bonding may increase the time spent by the electrophilic carbamate carbon near the catalytic residue Ser241, which should in turn favor the catalytic process. Since carbamate inhibitors of FAAH activity exert potent in vivo effects of therapeutic relevance,[29] including anxiolytic-like actions, a detailed knowledge of their SAR profile should be useful for further developments in this field.

Experimental Section

(a) Chemicals, Materials, and Methods. All reagents were purchased from Sigma-Aldrich in the highest quality commercially available. Solvents were RP grade unless otherwise indicated. Purification of the crude material obtained from the reactions, affording the desired products, was carried out by flash column chromatography on silica gel (Kieselgel 60, 0.040−0.063 mm, Merck). TLC analyses were performed on precoated silica gel on aluminum sheets (Kieselgel 60 F_{254}, Merck). Melting points were determined on a Büchi SMP-510 capillary melting point apparatus and are uncorrected. The structures of the unknown compounds were unambiguously assessed by MS, ^{1}H NMR, IR, and elemental analysis. EI-MS spectra (70 eV) were recorded with a Fisons Trio 1000 spectrometer. Only molecular ions (M^+) and base peaks are given. ^{1}H NMR spectra were recorded on a Bruker AC 200 spectrometer. Chemical shifts (δ scale) are reported in parts per million (ppm) relative to the central peak of the solvent. IR spectra were obtained on a Shimadzu FT-8300 or a Nicolet Atavar 360 FT spectometer. Absorbances are reported in ν (cm^{-1}). Elemental analyses were performed on a Carlo Erba analyzer. All products had satisfactory (within ±0.4 of theoretical values) C, H, N analysis results.

General Procedure for the Synthesis of Cyclohexylcarbamic Acid Aryl Esters (4a−c,e−i,k,l,m−y). To a stirred solution of the suitable aryl alcohol **3a**−**c**,**e**,**g**−**i**,**k**,**m**−**y** (1 mmol) in toluene (6 mL) were added Et_3N (0.006 g, 0.01 mL, 0.06 mmol) and c-$C_6H_{11}NCO$ (138 mg, 0.14 mL, 1.1 mmol). After the reactants were refluxed for 5 h (**4r**, 1 h; **4q**, 8 h; **4f**,**i**,**l**,**o**,**p**,**u**, 18 h), a further amount of c-$C_6H_{11}NCO$ [0.046 g, 0.05 mL, 0.37 mmol; 0.138 g, 0.14 mL, 1.1 mmol in the case of **4e**,**g**,**o**,**y** (no such further addition was necessary in the cases of **4f**,**i**,**p**,**q**,**r**,**v**,**w**)] was added, and the mixture was again reacted for 3 h. A third amount of c-$C_6H_{11}NCO$ (0.091 g, 0.09 mL, 0.726 mmol) was needed in the case of **4g**,**x**, the synthesis of which overall required 32 h. The mixture was then cooled and concentrated. Purification of the residue by column chromatography (cyclohexane/EtOAc 85:15 for **4b**,**g**,**h**,**k**,**o**; 4:6 for **4i**; 1:1 for **4f**,**p**; 55:45 for **4r**; 65:35 for **4l**,**u**; 7:3 for **4q**,**v**; 75:25 for **4s**,**x**; 8:2 for **4a**,**e**,**t**; 9:1 for **4m**,**p**; CH_2Cl_2 for **4n**; CH_2Cl_2/EtOAc 100:1 for **4y**; toluene/MeOH 95:5 and cyclohexane/EtOAc 7:3 for **4w**) and recrystallization gave **4a**−**c**,**e**−**i**,**k**,**l**,**m**−**w**.

Cyclohexylcarbamic Acid 4′-Trifluoromethylbiphenyl-3-yl Ester (4a). Colorless crystals were obtained. Yield: 77% (0.280 g). Mp: 147−148 °C (EtOH). MS (EI): *m*/*z* 238 (M^+), 69 (100). ^{1}H NMR ($CDCl_3$): δ 1.17−2.07 (m, 10H), 3.59 (m, 1H), 4.96 (d, 1H), 7.15−7.21 (m, 1H), 7.39−7.50 (m, 3H), 7.69 (s, 4H) ppm. IR (KBr): 3297, 1743, 1706 cm^{-1}. Anal. ($C_{20}H_{20}F_3NO_2$) C, H, N.

Cyclohexylcarbamic Acid 4′-Methylbiphenyl-3-yl Ester (4b). White crystals were obtained. Yield: 98% (0.303 g). Mp: 149−150 °C (MeOH). MS (EI): *m*/*z* 184, 61 (100). ^{1}H NMR ($CDCl_3$): δ 1.17−2.07 (m, 10H), 2.40 (s, 3H), 3.59 (m, 1H), 4.94 (br d, 1H), 7.10 (m, 1H), 7.22−7.51 (m, 7H) ppm. IR (Nujol): 3300, 1736, 1698, 1605, 1535, 1459 cm^{-1}. Anal. ($C_{22}H_{23}NO_2$) C, H, N.

Cyclohexylcarbamic Acid 4′-Carbamoylbiphenyl-3-yl Ester (4c). White crystals were obtained. Yield: 10% (0.034 g). Mp: 223−225 °C (EtOAc). MS (EI): *m*/*z* 213 (M^+), 197 (100). ^{1}H NMR ($CDCl_3$): δ 1.20−2.00 (m, 10H), 3.50 (m, 1H), 5.00 (br d, 1H), 5.60 (br s, 1H), 6.10 (br s, 1H), 7.15 (m, 1H), 7.40 (m, 3H), 7.65 (d, 2H), 7.90 (d, 2H) ppm. IR (KBr): 3379, 3331, 2926, 2849, 1705, 1627, 1578 cm^{-1}. Anal. ($C_{20}H_{22}N_2O_3$) C, H, N.

Cyclohexylcarbamic Acid 4′-Fluorobiphenyl-3-yl Ester (4e). White crystals were obtained. Yield: 80% (0.251 g). Mp: 131−133 °C (EtOH). MS (EI): *m*/*z* 313 (M^+), 188 (100). ^{1}H NMR ($CDCl_3$): δ 1.17−2.06 (m, 10H), 3.59 (br s, 1H), 4.97 (br d, 1H), 7.08−7.58 (m, 8H) ppm. IR (Nujol): 3316, 3294, 3055, 1747, 1709, 1587, 1579, 1534 cm^{-1}. Anal. ($C_{19}H_{20}FNO_2$) C, H, N.

Cyclohexylcarbamic Acid 4′-(3-Cyclohexylureidocarbonyl)biphenyl-3-yl Ester (4f). 4f was isolated as a side product of the synthesis of **4c**: white crystals. Yield: 11% (0.051 g). Mp: 184−186 °C (EtOAc). MS (EI): *m*/*z* 213 (M^+), 197 (100), 139, 115. ^{1}H NMR [$CD_3C(O)CD_3$]: δ 1.10−2.00 (m, 20H), 3.48 (m, 2H), 5.10 (br s, 1H), 6.70 (br s, 2H), 7.15 (m, 1H), 7.50 (m, 3H), 7.75 (d, 2H), 8.04 (d, 2H) ppm. IR (KBr): 3340, 3334, 3178, 2936, 2851, 1706, 1627, 1235 cm^{-1}. Anal. ($C_{27}H_{33}N_3O_4$) C, H, N.

Cyclohexylcarbamic Acid 3′-Trifluoromethylbiphenyl-3-yl Ester (4g). White crystals were obtained. Yield: 78% (0.283 g). Mp: 102−104 °C (EtOH). MS (EI): *m*/*z* 363 (M^+), 238 (100). ^{1}H NMR ($CDCl_3$): δ 1.17−2.07 (m, 10H), 3.60 (m, 1H), 4.96 (br d, 1H), 7.18 (m, 1H), 7.37−7.82 (m, 7H) ppm. IR (Nujol): 3298, 1742, 1709 cm^{-1}. Anal. ($C_{20}H_{20}F_3NO_2$) C, H, N.

Cyclohexylcarbamic Acid 3′-Methylbiphenyl-3-yl Ester (4h). White crystals were obtained. Yield: 78% (0.241 g). Mp: 133 °C (MeOH). MS (EI): *m*/*z* 309 (M^+), 184 (100). ^{1}H NMR ($CDCl_3$): δ 1.17−2.07 (m, 10H), 2.42 (s, 3H), 3.59 (m, 1H), 4.94 (br d, 1H), 7.08−7.40 (m, 8H) ppm. IR (Nujol): 3305, 1741, 1703, 1600, 1578, 1535, 1459 cm^{-1}. Anal. ($C_{20}H_{23}NO_2$) C, H, N.

Cyclohexylcarbamic Acid 3′-Carbamoylbiphenyl-3-yl Ester (4i). White crystals were obtained. Yield: 33% (0.112 g). Mp: 178 °C (EtOH) (sealed capillary tube). MS (EI): *m*/*z* 213 (100). ^{1}H NMR ($CDCl_3$): δ 1.17−1.43 (m, 6H), 1.76 (m, 2H), 2.04 (m, 2H), 3.57 (m, 1H), 4.97 (br d, 1H), 5.63 (br s, 1H), 6.14 (br s, 1H), 7.16 (m, 1H), 7.39−7.56 (m, 4H), 7.77 (m, 2H), 8.03 (s, 1H) ppm. IR ($CHCl_3$): 3301, 3142, 1693, 1666, 1627, 1604, 1573 cm^{-1}. Anal. ($C_{20}H_{22}N_2O_3$) C, H, N.

Cyclohexylcarbamic Acid 3′-Fluorobiphenyl-3-yl Ester (4k). White crystals were obtained. Yield: 77% (0.241 g). Mp: 128−130 °C (EtOH). MS (EI): *m*/*z* 313 (M^+), 188 (100). ^{1}H NMR ($CDCl_3$): δ 1.17−2.06 (m, 20H), 3.59 (m, 1H), 4.96 (br d, 1H), 6.99−7.44 (m, 8H) ppm. IR (Nujol): 3309, 3294, 1741, 1709, 1580, 1539 cm^{-1}. Anal. ($C_{19}H_{20}FNO_2$) C, H, N.

Cyclohexylcarbamic Acid 3′-Cyclohexylcarbamoyloxybiphenyl-3-yl Ester (4l). Isolated from the crude of synthesis of **4u**: white crystals. Yield: 20% (0.090 g). Mp: 214−216 °C (EtOH). MS (EI): *m*/*z* 186 (M^+), 157 (100). ^{1}H NMR ($CDCl_3$): δ 1.22−2.06 (m, 20H), 3.60 (m, 2H), 4.93 (br d, 2H), 7.10 (m, 2H), 7.30−7.50 (m, 6H) ppm. IR (KBr): 3423, 3312, 1765, 1644 cm^{-1}. Anal. ($C_{26}H_{32}N_2O_4S\cdot 0.1c\text{-}C_6H_{11}$) C, H, N.

Cyclohexylcarbamic Acid 3′-Phenoxybiphenyl-3-yl Ester (4m). White crystals were obtained. Yield: 74% (0.287 g). Mp: 142 °C (EtOH). MS (EI): *m*/*z* 262, 51 (100). ^{1}H NMR

($CDCl_3$): δ 1.17−2.06 (m, 10H), 3.58 (m, 1H), 4.93 (br d, 1H), 6.96−7.44 (m, 13H) ppm. IR (Nujol): 3305, 1741, 1709, 1578, 1540 cm^{-1}. Anal. ($C_{25}H_{25}NO_3$) C, H, N.

[1,1′;3′,1″]-Terphenyl-3-yl Carbamic Acid Cyclohexyl Ester (4n). White crystals were obtained. Yield: 74% (0.275 g). Mp: 177−179 °C (EtOAc). MS (EI): *m*/*z* 371 (M^+), 246 (100). ^1H NMR ($CDCl_3$): δ 1.17−2.06 (m, 10H), 3.60 (m, 1H), 4.95 (br d, 1H), 7.12−7.80 (m, 13H) ppm. IR (KBr): 3301, 2923, 2853, 1744, 1707 cm^{-1}. Anal. ($C_{25}H_{25}NO_2$) C, H, N.

Cyclohexylcarbamic Acid 3′-Benzylbiphenyl-3-yl Ester (4o). White crystals were obtained. Yield: 70% (0.270 g). Mp: 112−114 °C (EtOH). MS (EI): *m*/*z* 260 (M^+), 165 (100). ^1H NMR ($CDCl_3$): δ 1.20−1.50 (m, 6H), 1.60 (m, 2H), 2.00 (m, 2H), 3.58 (m, 1H), 4.04 (s, 2H), 4.93 (br d, 1H), 7.10−7.50 (m, 13H) ppm. IR (KBr): 3154, 1733, 1602, 1558, 1471 cm^{-1}. Anal. ($C_{26}H_{27}NO_2$) C, H, N.

Cyclohexylcarbamic Acid 3′-Propylbiphenyl-3-yl Ester (4p). Colorless crystals were obtained. Yield: 86% (0.290 g). Mp: 89−90 °C (petroleum ether). MS (EI): *m*/*z* 337 (M^+), 212 (100). ^1H NMR ($CDCl_3$): δ 0.97 (t, 3H), 1.16−1.79 (m, 10H), 2.04 (m, 2H), 2.65 (t, 2H), 3.59 (m, 1H), 4.94 (br d, 1H), 7.08−7.43 (m, 8H) ppm. IR (Nujol): 3310, 1736, 1703, 1546 cm^{-1}. Anal. ($C_{22}H_{27}NO_2$) C, H, N.

Cyclohexylcarbamic Acid 3′-Nitrobiphenyl-3-yl Ester (4q). Colorless needles. Yield: 75% (0.255 g). Mp: 138−142 °C (EtOH). MS (EI): *m*/*z* 215 (M^+), 141 (100). ^1H NMR ($CDCl_3$): δ 1.17−2.07 (m, 10H), 3.59 (m, 1H), 4.98 (br d, 1H), 7.21 (m, 1H), 7.45 (m, 3H), 7.61 (t, 1H), 7.92 (d, 1H), 8.22 (m, 1H), 8.45 (m, 1H) ppm. IR (Nujol): 3289, 1744, 1701, 1530, 1344 cm^{-1}. Anal. ($C_{19}H_{20}N_2O_4$) C, H, N.

Cyclohexylcarbamic Acid 3′-Sulfamoylbiphenyl-3-yl Ester (4r). White crystals which decompose starting from 115 °C (EtOAc/petroleum ether) were obtained. Yield: 26% (0.093 g). MS (EI): *m*/*z* 249 (M^+), 169 (100). ^1H NMR [$CD_3C(O)CD_3$]: δ 1.16−2.05 (m, 10H), 3.48 (br s, 1H), 6.68 (s, 2H), 6.77 (br d, 1H), 7.17 (d, 1H), 7.48 (m, 3H), 7.67 (t, 1H), 7.91 (d, 2H), 8.17 (s, 1H) ppm. IR (KBr): 3347, 3310, 3262, 1698, 1540 cm^{-1}. Anal. ($C_{19}H_{22}N_2O_4 \cdot 0.2C_6H_{14}$) C, H, N.

Cyclohexylcarbamic Acid 3′-Acetylbiphenyl-3-yl Ester (4s). Pearly scales were obtained. Yield: 72% (0.234 g). Mp: 130−132 °C (EtOH). MS (EI): *m*/*z* 212 (M^+), 197 (100). ^1H NMR ($CDCl_3$): δ 1.17−2.07 (m, 10H), 2.67 (s, 3H), 3.59 (m, 1H), 4.98 (br d, 1H), 7.17 (m, 1H), 7.39−7.58 (m, 4H), 7.79 (d, 1H), 7.95 (d, 1H), 8.17 (s, 1H) ppm. IR (Nujol): 3305, 1736, 1709, 1687, 1600, 1534 cm^{-1}. Anal. ($C_{21}H_{23}NO_3$) C, H, N.

Cyclohexylcarbamic Acid 3′-Cyanobiphenyl-3-yl Ester (4t). White crystals were obtained. Yield: 80% (0.257 g). Mp: 105−107 °C (EtOH) (sealed capillary tube). MS (EI): *m*/*z* 320 (M^+), 195 (100). ^1H NMR ($CDCl_3$): δ 1.22−2.07 (m, 10H), 3.58 (m, 1H), 4.97 (br d, 1H), 7.18 (m, 1H), 7.36−7.86 (m, 7H) ppm. IR (Nujol): 3305, 2235, 1741, 1703 cm^{-1}. Anal. ($C_{20}H_{20}N_2O_2$) C, H, N.

Cyclohexylcarbamic Acid 3′-Hydroxybiphenyl-3-yl Ester (4u). White crystals were obtained. Yield: 45% (0.148 g). Mp: 68−70 °C (EtOAc/hexane). MS (EI): *m*/*z* 186 (M^+), 157 (100). ^1H NMR ($CDCl_3$): δ 1.20−1.50 (m, 6H), 1.70 (m, 2H), 2.00 (m, 2H), 3.55 (m, 1H), 4.95 (br d, 1H), 5.10 (br s, 1H), 6.80 (m, 1H), 7.05 (m, 1H), 7.15 (m, 2H), 7.40 (m, 4H) ppm. IR (KBr): 3390, 2927, 2852, 1708, 1650 cm^{-1}. Anal. ($C_{19}H_{21}NO_3 \cdot 0.2$EtOAc) C, H, N.

Cyclohexylcarbamic Acid 3′-Hydroxymethylbiphenyl-3-yl Ester (4v). Colorless tablets were obtained. Yield: 64% (0.208 g). Mp: 137−140 °C (EtOH). MS (EI): *m*/*z* 325 (M^+), 153 (100). ^1H NMR ($CDCl_3$): δ 1.17−2.06 (m, 11H), 3.59 (m, 1H), 4.76 (s, 2H), 4.98 (br d, 1H), 7.13 (m, 1H), 7.34−7.54 (m, 6H), 7.59 (s, 1H) ppm. IR (KBr): 3446, 3272, 3061, 1709, 1595, 1540 cm^{-1}. Anal. ($C_{20}H_{23}NO_3$) C, H, N.

Cyclohexylcarbamic Acid 3′-(2-Hydroxyethyl)biphenyl-3-yl Ester (4w). White crystals were obtained. Yield: 13% (0.044 g). Mp: 106 °C (acetone/petroleum ether). MS (EI): *m*/*z* 214, 82 (100). ^1H NMR [$CD_3C(O)CD_3$]: δ 1.29−1.97 (m, 11H), 2.88 (t, 2H), 3.47 (m, 1H), 3.80 (t, 2H), 6.73 (br s, 1H), 7.06−7.54 (m, 8H) ppm. IR (Nujol): 3457, 3305, 3256, 1712, 1709, 1562, 1535 cm^{-1}. Anal. ($C_{21}H_{25}NO_3$) C, H, N.

Cyclohexylcarbamic Acid 3′-Benzyloxycarbonylaminobiphenyl-3-yl Ester (4x). A vitreous solid was obtained. Yield: 86% (0.382 g). MS (EI): *m*/*z* 319 (M^+), 91 (100). ^1H NMR ($CDCl_3$): δ 1.16−2.06 (m, 10H), 3.58 (m, 1H), 4.97 (br d, 1H), 5.23 (s, 2H), 6.80 (br s, 1H), 7.12 (m, 1H), 7.30−7.41 (m, 11H), 7.61 (s, 1H) ppm. IR (Nujol): 3308, 1709, 1606, 1537 cm^{-1}.

Cyclohexylcarbamic Acid 4′-Benzyloxycarbonylaminobiphenyl-3-yl Ester (4y). A white solid was obtained. Yield: 94% (0.418 g). Mp: 195−196 °C (EtOH). MS (EI): *m*/*z* 319 (M^+), 167. ^1H NMR ($CDCl_3$): δ 1.16−2.18 (m, 10H), 3.59 (m, 1H), 4.94 (br d, 1H), 5.23 (s, 2H), 6.74 (br s, 1H), 7.09 (m, 1H), 7.32−7.56 (m, 12H) ppm. IR (Nujol): 3332, 1703, 1600, 1540 cm^{-1}.

General Procedure for the Synthesis of 3′-(or 4′-)-Substituted-3-methoxybiphenyls (8a−e,g,h,j,k,m−o,q,9) and 3-Substituted-1-hydroxybiphenyls (3i,r−w). To a stirred solution of the opportune halobenzene **6a**−**e**,**g**−**k**,**m**−**o**,**q**−**w**,**7** (15.5 mmol) in toluene (100 mL) were added $Pd(PPh_3)_4$ (0.726 g, 0.63 mmol), a solution of Na_2CO_3 (10.323 g, 97.37 mmol) in H_2O (50 mL), and a solution of the suitable 3-methoxyphenyl boronic acid (**5a**) (31 mmol) or 3-hydroxyphenyl boronic acid (**5b**) (15.5 mmol) in EtOH (44 mL) under N_2 atmosphere. The mixture was vigorously stirred under reflux for the opportune time (1 h for **3r**−**t**,**v**,**w**,**8b**,**h**,**j**,**9**; 2 h for **3i**,**8a**,**m**,**q**; 3 h for **3u**,**8c**−**e**,**g**,**k**,**n**,**o**) and cooled, H_2O was added, and the mixture was extracted with CH_2Cl_2 (EtOAc in the case of **3i**,**r**,**t**,**w**,**8d**,**m**; the acidification of the aqueous phase with 2 N HCl was necessary for **3r**). The combined organic layers were dried (Na_2SO_4) and concentrated. Purification of the residue by column chromatography (cyclohexane/EtOAc 200:1 for **8m**; 98:2 for **8b**,**h**,**n**,**o**; 97:3 for **8e**; 95:5 for **8g**,**k**; 85:15 for **8q**,**9**; 8:2 for **8a**; 75:25 for **3s**; 7:3 for **3u**,**8j**; 65:35 for **3t**,**v**; 6:4 for **8d**; 1:1 for **3r**; 7:3 to 6:4 to 1:1 for **3w**; 2:8 for **3i**; EtOAc for **8c**) gave **8a**,**d**,**e**,**g**,**h**,**j**,**k**,**m**−**o**,**9** and **3r**,**s**,**v**,**w** as oils and **8b**,**c**,**q** and **3i**,**t**,**u** as solids.

3-Methoxy-4′-trifluoromethylbiphenyl (8a).[30] A colorless oil was obtained. Yield: 95% (3.714 g). MS (EI): *m*/*z* 252 (M^+, 100). The ^1H NMR spectrum is according to literature.[30]

3-Methoxy-4′-methylbiphenyl (8b).[31] White crystals were obtained. Yield: 94% (2.889 g). Mp: 75−76 °C (MeOH). MS (EI): *m*/*z* 198 (M^+), 69 (100). ^1H NMR ($CDCl_3$): δ 2.40 (s, 3H), 3.87 (s, 3H), 6.88 (m, 1H), 7.11−7.52 (m, 7H) ppm. IR (neat): 3055, 3006, 1611, 1584, 1567, 1519, 1486 cm^{-1}.

3′-Methoxybiphenyl-4-carboxylic Acid Amide (8c). White crystals were obtained. Yield: 55% (1.937 g). Mp: 198−200 °C (EtOH). MS (EI): *m*/*z* 227 (M^+), 199 (100). ^1H NMR ($CDCl_3$): δ 3.90 (s, 3H), 5.60 (br s, 1H), 6.10 (br s, 1H), 6.95 (dd, 1H), 7.17 (m, 2H), 7.37 (t, 1H), 7.67 (d, 2H), 7.89 (d, 2H) ppm.

3′-Methoxybiphenyl-4-yl Amine (8d).[32] A yellow oil was obtained. Yield: 57% (1.760 g). MS (EI): *m*/*z* 199 (M^+, 100), 156. ^1H NMR ($CDCl_3$): δ 3.86 (s and br s, 5H), 6.74−7.44 (m, 8H) ppm. IR (neat): 3457, 3370, 3218, 3033, 3001, 2952, 2936, 1600 cm^{-1}.

4′-Fluoro-3-methoxybiphenyl (8e).[33] A colorless oil was obtained. Yield: 62% (1.943 g). MS (EI): *m*/*z* 202 (M^+), 133 (100).

3-Methoxy-3′-trifluoromethylbiphenyl (8g). A colorless oil was obtained. Yield: 93% (3.636 g). MS (EI): *m*/*z* 252 (M^+), 69 (100). ^1H NMR ($CDCl_3$): δ 3.89 (s, 3H), 6.93−7.84 (m, 8H) ppm. IR (neat): 3071, 3001, 2952, 2838, 1600, 1578, 1486, 1464 cm^{-1}.

3-Methoxy-3′-methylbiphenyl (8h).[34] A pink oil was obtained. Yield: 95% (2.919 g). MS (EI): *m*/*z* 198 (M^+), 155 (100).

3′-Methoxybiphenyl-3-yl Amine (8j). A yellow oil was obtained. Yield: 94% (2.903 g). MS (EI): *m*/*z* 199 (M^+, 100), 156. ^1H NMR ($CDCl_3$): δ 3.87 (s and br s, 5H), 6.69 (s, 1H), 6.86−7.38 (m, 7H) ppm. IR (neat): 3457, 3365, 3213, 3033, 2963, 2936, 1622, 1600, 1578 cm^{-1}.

3′-Fluoro-3-methoxybiphenyl (8k).[35] A pale yellow oil was obtained. Yield: 83% (2.602 g). MS (EI): *m*/*z* 202 (M^+, 100). ^1H NMR ($CDCl_3$): δ 3.89 (s, 3H), 6.91−7.43 (m, 8H) ppm. IR (neat): 3071, 2995, 2963, 2936, 2838, 1608, 1587 cm^{-1}.

3-Methoxy-3′-phenoxybiphenyl (8m). A light yellow oil was obtained. Yield: 42% (1.800 g). MS (EI): *m/z* 276 (M^+, 100). ^{1}H NMR ($CDCl_3$): δ 3.87 (s, 3H), 6.89−7.45 (m, 13H) ppm. IR (neat): 3061, 3033, 2995, 2925, 2849, 2832, 1595, 1573 cm^{-1}.

3-Methoxy-[1,1′;3,1″]terphenyl (8n). An opaque white oil was obtained. Yield: 97% (3.914 g). MS (EI): *m/z* 260 (M^+), 77 (100). ^{1}H NMR ($CDCl_3$): δ 3.89 (s, 3H), 6.92−7.82 (m, 13H) ppm. IR (film): 3061−2834 cm^{-1}.

3′-Benzyl-3-methoxybiphenyl (8o). An amber oil was obtained. Yield: 95% (4.040 g). MS (EI): *m/z* 274 (M^+), 165 (100). ^{1}H NMR ($CDCl_3$): δ 3.87 (m, 12H), 6.90 (dd, 1H), 4.06 (s, 2H), 3.87 (s, 3H) ppm. IR (Nujol): 3154, 2962, 1599, 1468, 1381 cm^{-1}.

3′-Methoxy-3-nitrobiphenyl (8q). Gray sheaves were obtained. Yield: 93% (3.304 g). Mp: 75−76 °C (EtOH) [lit.: 77−78 °C (EtOH)].[36] MS (EI): *m/z* 229 (M^+, 100), 168. IR (Nujol): 1605, 1584, 1567, 1524, 1345 cm^{-1}. The ^{1}H NMR spectrum is according to literature.[36]

3′-Methoxybiphenyl-3-carbaldehyde (9). An opaque yellow oil was obtained. Yield: 82% (2.698 g). MS (EI): *m/z* 212 (M^+), 168 (100). The ^{1}H NMR and IR spectra are in agreement with literature.[37]

3′-Hydroxybiphenyl-3-carboxylic Acid Amide (3i). A sand-colored solid was obtained. Yield: 98% (3.239 g). Mp: 148−151 °C (after digestion with *i*-Pr_2O). MS (EI): *m/z* 213 (M^+, 100). ^{1}H NMR [$CDCl_3$/$(CD_3)_2SO$]: δ 6.06 (br s, 1H), 6.59 (m, 1H), 6.85 (m, 2H), 7.01 (t, 1H), 7.23 (t, 1H), 7.35 (br s, 1H), 7.45 (m, 1H), 7.60 (m, 1H), 7.88 (s, 1H), 8.80 (s, 1H) ppm. IR (Nujol): 3314, 3141, 1699, 1630, 1607, 1577 cm^{-1}.

3′-Hydroxybiphenyl-3-sulfonic Acid Amide (3r).[38] A pale yellow oil was obtained. Yield: 86% (3.323 g). MS (EI): *m/z* 249 (M^+), 169 (100). ^{1}H NMR [$CD_3C(O)CD_3$]: δ 6.65 (br s, 2H), 6.90 (dd, 1H), 7.15 (m, 2H), 7.30 (t, 1H), 7.60 (t, 1H), 7.85 (m, 1H), 8.12 (m, 1H), 8.60 (br s, 1H) ppm. IR (Nujol): 3360, 3100, 2933, 2855, 1693, 1599 cm^{-1}.

1-(3′-Hydroxybiphenyl-3-yl)ethanone (3s). A yellowish-gray oil was obtained. Yield: 69% (2.270 g). MS (EI): *m/z* 212 (M^+), 197 (100). ^{1}H NMR ($CDCl_3$): δ 2.68 (s, 3H), 5.18 (s, 1H), 6.88 (m, 1H), 7.12 (t, 1H), 7.20 (d, 1H), 7.35 (t, 1H), 7.54 (t, 1H), 7.79 (d, 1H), 7.95 (d, 1H), 8.18 (t, 1H) ppm. IR (Nujol): 3348, 1660, 1616, 1600, 1584 cm^{-1}.

3′-Hydroxybiphenyl-3-carbonitrile (3t).[39] White crystals were obtained (lit.: liquid).[39] Yield: 90% (2.723 g). Mp: 107−108 °C (petroleum ether). MS (EI): *m/z* 195 (M^+), 140 (100). ^{1}H NMR and IR spectra are according to literature.[39]

Biphenyl-3,3′-diol (3u).[40] A yellow solid was obtained. Yield: 89% (2.569 g). MS (EI): *m/z* 186 (M^+, 100). ^{1}H NMR ($CDCl_3$): δ 4.97 (br s, 2H), 6.84 (ddd, 2H), 7.05 (m, 2H), 7.16 (ddd, 2H), 7.32 (t, 2H) ppm. IR (neat): 3465, 3270, 2922, 2852, 1606, 1577, 1433, 1181 cm^{-1}.

3′-Hydroxymethylbiphenyl-3-ol (3v). A pale green oil was obtained. Yield: 92% (2.855 g). MS (EI): *m/z* 200 (M^+), 153 (100). ^{1}H NMR ($CDCl_3$): δ 1.83 (br s, 1H), 4.78 (s, 2H), 5.12 (s, 1H), 6.84 (m, 1H), 7.07 (m, 1H), 7.18 (d, 1H), 7.28−7.53 (m, 4H), 7.59 (s, 1H) ppm. IR (neat): 3338, 2930, 2881, 1703, 1595, 1578 cm^{-1}.

3′-(2-Hydroxyethyl)biphenyl-3-ol (3w). A pale yellow oil was obtained. Yield: 63% (2.092 g). MS (EI): *m/z* 214 (M^+), 183 (100). ^{1}H NMR [$CD_3C(O)CD_3$]: δ 2.09 (s, 1H), 2.89 (t, 2H), 3.80 (t, 2H), 6.83 (m, 1H), 7.09−7.50 (m, 7H), 8.44 (s, 1H) ppm. IR (neat): 3327, 1600, 1578 cm^{-1}.

General Procedure for the Synthesis of 3′-(or 4′-)-Substituted Biphenyl-3-ols (3a,b,d,e,g,h,j,k,m,n,p). The opportune methoxybenzene **8a**,**b**,**d**,**e**,**g**,**h**,**j**,**k**,**m**,**n**,**p** (3.5 mmol) and 57% HI (4.375 mL) were refluxed with vigorous stirring (2 h for **3a**,**b**,**d**,**h**,**j**,**k**,**n**; 3 h for **3e**,**m**,**p**; 7.5 h for **3g**). The mixture was cooled, diluted with H_2O, neutralized with 2 N $NaHCO_3$, and extracted with EtOAc (CH_2Cl_2 in the case of **3b**,**h**,**n**). The combined organic phases were dried (Na_2SO_4) and concentrated. Purification of the residue by column chromatography (cyclohexane/EtOAc 9:1 for **3a**; 9:1 to 8:2 for **3b**,**h**,**n**,**p**; 8:2 for **3e**,**k**,**m**; 7:3 for **3g**; 6:4 for **3j**; 1:1 for **3d**) gave **3a**,**b**,**d**,**e**,**g**,**j**,**n** as solids and **3h**,**m**,**h**,**k**,**p** as oils.

4′-Trifluomethylbiphenyl-3-ol (3a). Colorless needles were obtained. Yield: 70% (0.584 g). Mp: 73−75 °C (petroleum ether). MS (EI): *m/z* 238 (M^+), 69 (100). ^{1}H NMR ($CDCl_3$): δ 4.91 (s, 1H), 6.88 (m, 1H), 7.08 (m, 1H), 7.18 (d, 1H), 7.35 (t, 1H), 7.69 (s, 4H) ppm. IR (Nujol): 3280, 1616, 1600, 1590, 1573 cm^{-1}.

4′-Methylbiphenyl-3-ol (3b). White crystals were obtained. Yield: 85% (0.548 g). Mp: 74−75 °C (cyclohexane). MS (EI): *m/z* 184 (M^+), 165 (100). ^{1}H NMR ($CDCl_3$): δ 2.40 (s, 3H), 4.87 (s, 1H), 6.81 (m, 1H), 7.06 (t, 1H), 7.14−7.50 (m, 6H) ppm. IR (Nujol): 3289, 1589, 1567, 1464 cm^{-1}.

4′-Aminobiphenyl-3-ol (3d). A light-brown solid was obtained. Yield: 77% (0.499 g). Mp: 163−165 °C (EtOAc/petroleum ether). MS (EI): *m/z* 185 (M^+), 156 (100). ^{1}H NMR ($CDCl_3$): δ 3.81 (br s, 2H), 6.63 (m, 3H), 6.89 (m, 2H), 7.09 (t, 1H), 7.26 (m, 2H), 8.57 (s, 1H) ppm. IR (Nujol): 3382, 3313, 1596, 1579 cm^{-1}.

4′-Fluorobiphenyl-3-ol (3e).[41] An amorphous solid was obtained. Yield: 53% (0.349 g). MS (EI) is according with literature.[41]

3′-Trifluomethylbiphenyl-3-ol (3g). A white solid was obtained. Yield: 82% (0.684 g). Mp: 63−66 °C (petroleum ether). MS (EI): *m/z* 238 (M^+, 100). ^{1}H NMR ($CDCl_3$): δ 4.97 (s, 1H), 6.88 (m, 1H), 7.08 (m, 1H), 7.16−7.82 (m, 6H) ppm. IR (Nujol): 3341, 1618, 1598, 1583 cm^{-1}.

3′-Methylbiphenyl-3-ol (3h).[42] A brown oil was obtained. Yield: 90% (0.580 g). MS (EI): *m/z* 184 (M^+, 100). ^{1}H NMR ($CDCl_3$): δ 2.40 (s, 3H), 4.86 (s, 1H), 6.82 (m, 1H), 7.06 (t, 1H), 7.15−7.40 (m, 6H) ppm. IR (neat): 3609, 3365, 3115, 3039, 2919, 1709, 1611, 1578, 1475 cm^{-1}.

3′-Aminobiphenyl-3-ol (3j). A light-brown powder was obtained. Yield: 56% (0.363 g). Mp: 154−159 °C (acetone/petroleum ether). MS (EI): *m/z* 185 (M^+), 63 (100). ^{1}H NMR ($CDCl_3$): δ 3.77 (br s, 2H), 6.58 (m, 1H), 6.73−7.20 (m, 7H), 8.59 (s, 1H) ppm. IR (neat): 3381, 3305, 1586 cm^{-1}.

3′-Fluorobiphenyl-3-ol (3k).[35] A yellow oil was obtained. Yield: 97% (0.639 g). MS (EI): *m/z* 188 (M^+, 100), 159. ^{1}H NMR ($CDCl_3$): δ 5.74 (s, 1H), 6.89 (m, 1H), 7.01−7.45 (m, 7H) ppm. IR (neat): 3354, 3066, 2925, 2854, 1611, 1578 cm^{-1}.

3′-Phenoxybiphenyl-3-ol (3m). A light-brown oil was obtained. Yield: 95% (0.872 g). MS (EI): *m/z* 262 (M^+), 77 (100). ^{1}H NMR ($CDCl_3$): δ 4.81 (s, 1H), 6.80−7.44 (m, 13H) ppm. IR (film): 3381, 3061, 3033, 2925, 2849, 1703, 1600, 1573 cm^{-1}.

[1,1′;3,1″]-Terphenyl-3-ol (3n). Gray crystals were obtained. Yield: 97% (0.836 g). Mp: 93−95 °C (cyclohexane). MS (EI): *m/z* 246 (M^+, 100). ^{1}H NMR ($CDCl_3$): δ 4.97 (s, 1H), 6.83−7.81 (m, 13H) ppm. IR (KBr): 3470, 3391, 3046 cm^{-1}.

3′-Propylbiphenyl-3-ol (3p). A colorless oil was obtained. Yield: 65% (0.483 g). MS (EI): *m/z* 212 (M^+, 100).

General Procedure for the Synthesis of 3′-(or 4′-)-Substituted Biphenyl-3-ols (3c,o,q). To a stirred, cooled (0 °C), 1 M solution of BBr_3 in CH_2Cl_2 (7.8 mL) was added a solution of the opportune methoxybenzene **8c**,**o**,**q** (3 mmol) in dry CH_2Cl_2 (35 mL) under N_2 atmosphere. The mixture was stirred at room temperature (1 h for **3q**; 5 h for **3c**; 18 h for **3o**), then quenched with 2N Na_2CO_3, and extracted with EtOAc. The combined organic phases were dried (Na_2SO_4) and concentrated. Purification of the residue by column chromatography (cyclohexane/EtOAc 8:2 for **3c**,**o**; 7:3 for **3q**) gave **3c**,**q** as solids and **3o** as an oil.

3′-Hydroxybiphenyl-4-carboxylic Acid Amide (3c). White crystals were obtained. Yield: 76% (0.486 g). Mp: 187−189 °C (EtOAc/petroleum ether). MS (EI): *m/z* 213 (M^+), 139 (100). ^{1}H NMR [$CD_3C(O)CD_3$]: δ 6.70 (br s, 1H), 6.87 (m, 1H), 7.17 (m, 2H), 7.30 (m, 1H), 7.54 (br s, 1H), 7.70 (d, 2H), 8.01 (d, 2H), 8.62 (br s, 1H) ppm. IR (KBr): 3449, 3339, 3291, 3209, 1652, 1610, 1413, 1299 cm^{-1}.

3′-Benzylbiphenyl-3-ol (3o). An amber oil was obtained. Yield: 75% (0.458 g). MS (EI): *m/z* 260 (M^+, 100). ^{1}H NMR ($CDCl_3$): δ 4.05 (s, 2H), 6.80 (dd, 1H), 7.10−7.50 (m, 12H) ppm. IR (KBr): 3376, 3028, 2923 cm^{-1}.

3′-Nitrobiphenyl-3-ol (3q).[43] Yellow crystals were obtained. Yield: 90% (0.581 g). Mp: 118−119 °C (EtOAc/

petroleum ether) [lit.: 117 °C (benzene)].[43] MS (EI): *m*/*z* 215 (M^+, 100), 168. ^{1}H NMR ($CDCl_3$): δ 4.95 (s, 1H), 6.92 (m, 1H), 7.12 (t, 1H), 7.23 (d, 1H), 7.38 (t, 1H), 7.62 (t, 1H), 7.91 (d, 1H), 8.22 (d, 1H), 8.45 (t, 1H) ppm. IR (Nujol): 3500, 3093, 1605, 1535, 1508, 1344 cm^{-1}.

Synthesis of (3′-Hydroxybiphenyl)carbamic Acid Benzyl Esters (3x,y). To a stirred solution of $NaHCO_3$ (0.157 g, 1.87 mmol) in H_2O (3 mL) were added the opportune biphenyl-3-ol **3d**,**j** (0.315 g, 1.7 mmol) and benzyl chloroformate (0.290 g, 1.7 mmol, 0.24 mL). The mixture was stirred 16 h, and then a further amount of benzyl chloroformate (one drop) was added. The mixture was stirred for other 4 h and then extracted with EtOAc. The combined organic layers were dried over Na_2SO_4 and evaporated. Purification of the residue by column chromatography (cyclohexane/EtOAc 7:3) gave **3x** as an oil and **3y** as a solid.

(3′-Hydroxybiphenyl-3-yl)carbamic Acid Benzyl Ester (3x). A white oil was obtained. Yield: 94% (0.510 g). MS (EI): *m*/*z* 319 (M^+), 91 (100). ^{1}H NMR ($CDCl_3$): δ 4.72 (s, 1H), 5.23 (s, 2H), 6.81−7.62 (m, 14H) ppm. IR (Nujol): 3386, 3321, 1709, 1611, 1584, 1546 cm^{-1}.

(3′-Hydroxybiphenyl-4-yl)carbamic Acid Benzyl Ester (3y). White crystals were obtained. Mp: 175−176 °C (EtOAc/petroleum ether). Yield: 66% (0.358 g). MS (EI): *m*/*z* 319 (M^+), 91 (100). ^{1}H NMR ($CDCl_3$): δ 4.93 (s, 1H), 5.23 (s, 2H), 6.73 (br s, 1H), 6.80 (m, 1H), 7.04 (t, 1H), 7.14 (m, 1H), 7.30−7.59 (m, 10H) ppm. IR (Nujol): 3381, 1675, 1599, 1538 cm^{-1}.

Synthesis of Cyclohexylcarbamic Acid Aminobiphenyl-3-yl Esters (4d,j). To a stirred solution of the convenient carbamic acid biphenyl ester **4x**,**y** (0.356 g, 0.8 mmol) in THF (15 mL) were added MeOH (0.26 mL) and 10% Pd/C (0.039 g). The mixture was hydrogenated at 3 atm at 65 °C for 24 h, cooled, filtered on Celite, and concentrated. Purification of the residue by column chromatography (cyclohexane/EtOAc 6:4) gave **4d**,**j** as solids.

Cyclohexylcarbamic Acid 4′-Aminobiphenyl-3-yl Ester (4d). An off-white powder was obtained. Yield: 42% (0.106 g). Mp: 121−125 °C (acetone/petroleum ether). MS (EI): *m*/*z* 310 (M^+), 185 (100). ^{1}H NMR ($CDCl_3$): δ 1.20−2.05 (m, 10H), 2.80 (m, 2H), 3.60 (m, 1H), 4.97 (br d, 1H), 6.74 (m, 2H), 7.05 (m, 1H), 7.29−7.42 (m, 5H) ppm. IR (Nujol): 3457, 3403, 3294, 1747, 1709, 1627, 1605 cm^{-1}. Anal. [$C_{19}H_{22}N_2O_2$·0.10MeC(O)Me] C, H, N.

Cyclohexylcarbamic Acid 3′-Aminobiphenyl-3-yl Ester (4j). Light yellow crystals were obtained. Yield: 83% (0.206 g). Mp: 129−134 °C (EtOH). MS (EI): *m*/*z* 310 (M^+), 185 (100). ^{1}H NMR ($CDCl_3$): δ 1.22−2.06 (m, 10H); 3.59 (m, 1H); 3.75 (m, 2H); 4.96 (br d, 1H); 6.68 (m, 1H); 6.90−7.41 (m, 7H) ppm. IR (Nujol): 3466, 3388, 3297, 1740, 1707, 1609, 1581, 1540 cm^{-1}. Anal. ($C_{19}H_{22}N_2O_2$) C, H, N.

Synthesis of 3-Methoxy-3′-propenylbiphenyl (11). To a stirred solution of ethyltriphenylphosphonium bromide (**10**) (4.529, 12.2 mmol) in dry THF (122 mL) was added 2M *n*-BuLi 2M in hexane (6.1 mL) under N_2 atmosphere at 0 °C. The mixture was stirred for 2 h and cooled (−78 °C), and then **9** (2.589 g, 12.2 mmol) was added. The mixture was allowed to reach room temperature, refluxed for 20 h, and cooled. H_2O was cautiously added, and the mixture was extracted with EtOAc. The combined organic layers were dried (Na_2SO_4) and evaporated. Purification of the residue by column chromatography (cyclohexane/EtOAc 9:1) gave **11** as a colorless oil. Yield: 41% (1.122 g). MS (EI): *m*/*z* 224 (M^+, 100), 181, 165, 115.

Synthesis of 3-Methoxy-3′-propylbiphenyl (8p). 11 (1.009 g, 4.8 mmol) was dissolved in EtOH (60 mL) and, after addition of PdO_2 (0.103 g, 0.843 mmol), hydrogenated at room temperature at 3 atm for 3 h. The mixture was filtered on Celite and concentrated. Purification of the residue by column chromatography (cyclohexane/EtOAc 95:5) gave **8p** as a yellow oil. Yield: 76% (0.826 g). MS (EI): *m*/*z* 226 (M^+), 197 (100). ^{1}H NMR ($CDCl_3$): δ 0.98 (t, 3H), 1.70 (m, 2H), 2.66 (t, 2H), 3.88 (s, 3H), 6.91 (m, 1H), 7.14−7.41 (m, 7H) ppm. IR (neat): 3060, 3029, 2990, 2959, 2920, 2870, 2833, 1596, 1573 cm^{-1}.

Synthesis of Cyclohexylcarbamic Acid 3′-Aminomethylbiphenyl-3-yl Ester (4z). To a stirred solution of **4t** (0.175 g, 0.54 mmol) in THF (5 mL) was added Raney nickel. The mixture was hydrogenated at 4 atm at 65 °C for 3 h, cooled, filtered on Celite (DANGER! Raney nickel is pyrophoric if allowed to dry), and concentrated. Purification of the residue by column chromatography (cyclohexane/EtOAc 6:4) and recrystallization gave **4z** as white crystals. Yield: 30% (0.053 g). Mp: 154−157 °C (with decomposition) (EtOAc/petroleum ether) (sealed capillary tube). MS (EI): *m*/*z* 324 (M^+), 82 (100). ^{1}H NMR [$CD_3C(O)CD_3$]: δ 1.04−1.89 (m, 10H), 3.55 (m, 1H), 4.38 (d, 2H), 5.55 (br d, 1H), 5.97 (br s, 1H), 6.83 (m, 1H), 7.10−7.55 (m, 7H), 8.64 (s, 1H) ppm. IR (Nujol): 3365, 3338, 1599, 1573 cm^{-1}. Anal. ($C_{20}H_{24}N_2O_2$) C, H, N.[44]

Synthesis of 3-Bromobenzoic Acid Amide (6i). To a stirred solution of 3-bromobenzonitrile (3.555 g, 19.5 mmol) in dioxane (74 mL) were added $NaBO_3$·$4H_2O$ (8.269 g, 53.74 mmol) and H_2O (74 mL). The mixture was stirred at 80 °C for 16 h, cooled, H_2O was added and the mixture was extracted with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and evaporated. Purification of the residue by column chromatography (hexane/EtOAc 2:8) and recrystallization gave **6i** as colorless tablets. Yield: 80% (3.12 g). Mp: 156−157 °C (EtOH) (lit. 156 °C).[45] MS (EI): *m*/*z* 199 (M^+); 183 (100).

(b) Pharmacology. Membrane fractions were prepared from brain homogenates, and FAAH activity was assayed using [^{3}H]anandamide (anandamide[ethanolamine-^{3}H], American Radiolabeled Chemicals, ARC, St. Louis, MO, 60 Ci/mmol) as a substrate. Rat brain membranes (50 μg protein) were incubated for 30 min at 37 °C in buffer containing [^{3}H]-anandamide and varying concentrations of test compounds. At the end of the incubation period, we stopped the reactions with a mixture of chloroform/methanol and measured [^{3}H]-ethanolamine in the aqueous phase by liquid scintillation counting.[19,20] [^{3}H]Anandamide transport assays were conducted in human astrocytoma cells, preincubating cells with inhibitors for 10 min at 37 °C, prior to exposure to [^{3}H]-anandamide for 4 min.[7] CB_1 and CB_2 binding assays were conducted in rat cerebellar membranes (27000*g*) and CB_2-overexpressing CHO cells (purchased from Receptor Biology-Perkin-Elmer, Wellesley, MA), respectively, using [^{3}H]WIN-55212-2 (NEN-Dupont, Boston, MA, 40−60 Ci/mmol, 10 nM) as a ligand.[8,46] Cholinesterase assays were conducted with a commercial kit (Sigma, St. Louis, MO), using purified enzymes (electric eel acetylcholinesterase type V-S and horse serum cholinesterase, both from Sigma, St. Louis, MO) and following vendor's instructions.

(c) QSAR. MRA calculations were performed with an Excel (Microsoft Co., version 97) spreadsheet, employing the built-in statistical functions and automated macro procedures. Substituent properties were parametrized by the variables π, π^2 σ_m, *F*, *R*, MR, *L*, B_1, B_5. When available, their values were taken from the van de Waterbeemd data set;[47] for the benzyl, hydroxyethyl, and aminomethyl substituents they were taken from the Hansch collection;[48] π and MR values for the cyclohexylcarbamoyloxy group (**4l**) were estimated by the Chem Prop module in Chem Draw,[49] and those of the electronic variables from similar alkylaminocarbonyloxy groups in the Hansch collection. MRA models were calculated for all the possible combinations of maximum five variables. Standard deviation of the errors in prediction (SDEP) and the relative predictivity parameter, q^2, were calculated by cross-validation, omitting one compound at a time from the set, according to the leave-one-out technique (LOO).[50]

(d) Molecular Modeling. Molecular models were built, refined, and analyzed by Sybyl version 6.8.[51] Energy calculations were performed employing the Merck molecular force field (MMFF94s for geometry optimization and MMFF94 for molecular dynamics),[52] implemented in Sybyl, with the dielectric set constant to 1. FAAH structure coordinates were taken from those of the covalent adduct with a MAP chain, reported in the Protein Data Bank[53] (PDB code: 1MT5). The first structure refinement (addition of missing side chains and hydrogens) was done by the Biopolymer module, and it was

followed by a visual inspection of histidine tautomerism, which was modified to maximize the number of possible hydrogen bonds. The geometry of added atoms was then relaxed by energy minimization to a gradient of 0.1 kcal/(mol·Å), the MAP atoms removed, and the hydrogen atoms at catalytic site residues were reassigned to get a hydrogen bond between Ser241 and Ser217, and one between Ser217 and the NH_2 group of Lys142. The inhibitors were then fitted into the enzyme channel, optimizing their position and conformation first by the Dock_minimize procedure, then by energy minimization of the complex, allowing movements of the residues at maximum 8 Å from the inhibitor. Molecular dynamics simulations (step size of 1 fs) started with seven 3000-step heating cycles, gradually raising *T* from 0 to 310 K and continued with 200000 fs of simulation; after 100000 fs of equilibration, a snapshot of the trajectory was saved every 500 fs for subsequent analysis. The surface of the enzyme channel shown in Figures 2 and 4 was built by the MOLCAD module in Sybyl.

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Supporting Information Available: Table of elemental analytical data. This material is available free of charge via the Internet at http://pubs.acs.org.

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