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Synthesis and Structure–Activity Relationships of FAAH Inhibitors: Cyclohexylcarbamic Acid Biphenyl Esters with Chemical Modulation at the Proximal Phenyl Ring

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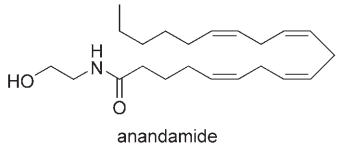
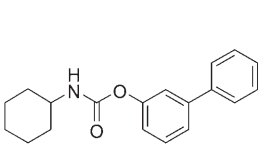
Fatty acid amide hydrolase (FAAH) is a serine hydrolase that catalyzes the intracellular hydrolysis of fatty acid ethanolamides such as anandamide and oleoylethanolamide. Targeting this enzyme may have important therapeutic potentials owing to the multiple physiological roles of these amides. Cyclohexylcarbamic acid biphenyl-3-yl ester (URB524) was one of the most promising FAAH inhibitors so far described. We report the modulation of the electronic and steric features of the proximal phenyl ring of this compound by introducing a series of substituents at the ortho and para positions. pIC_{50} values were found to correlate with molecular features thought to be involved in the recognition step such as steric hindrance and hydrogen-bonding ability. Derivatives with small polar groups at the para position of the proximal phenyl ring were slightly better FAAH inhibitors than the parent compound URB524.

Introduction

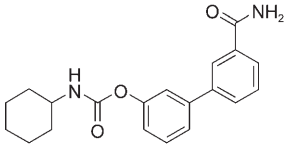
The endogenous cannabinoid system has been implicated recently in the regulation of many physiologic processes.[1] This signaling system involves two G-protein-coupled receptors referred to as CB_1 and CB_2, at least two lipid ligands: *N*-arachidonoylethanolamine (anandamide) and 2-arachidonoylglycerol (2-AG), and a number of proteins responsible for the synthesis, transport, and catabolism of the above-mentioned ligands.[1] Evidence suggests that pharmacological modulation of the endocannabinoid system may lead to innovative therapies. In particular, it has been suggested that an increase in the level of endogenous cannabinoids by blockade of their catabolizing enzymes, such as monoacylglycerol lipase and fatty acid amide hydrolase (FAAH), may exert favorable biologic effects while avoiding the disadvantages of the global activation of cannabinoid receptors through exogenous direct agonists.[2–4]



anandamide



URB524



URB597

In support of this idea, reports of different classes of FAAH inhibitors have been published over the last decade. They include fatty acid derivatives such as sulfonyl fluorides and fluorophosphonate,[5] α-ketoesters, α-ketoamides, trifluoromethylketones, and acyl heterocycles.[6] Moreover, nonlipid inhibitors have also been developed including carbamate derivatives[7,8] and keto heterocycles.[9]

We recently discovered a class of FAAH inhibitors based on a carbamate template which produce anxiolytic-like effects[8] and which normalize blood pressure in rats[10] at doses that do not elicit the classical signs of cannabinoid intoxication (such as catalepsy and hypothermia, for example). Structure–activity relationship (SAR) investigations led to the identification of cyclohexylcarbamic acid biphenyl-3-yl ester (URB524) as a lead compound for further optimization studies.[11] In a first step of structural modulation,[12] we observed that the introduction of hydrophilic residues (carbamoyl, hydroxymethyl, and hydroxy

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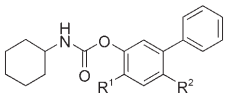
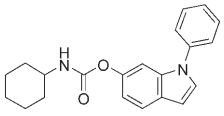
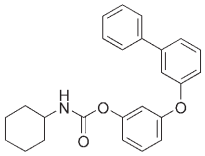
groups) at the *meta* position of the distal phenyl ring yielded FAAH inhibitors with potency in the low nanomolar concentration range, such as URB597, the pharmacological properties of which have been described.[13]

Herein, we report the syntheses and SAR for a novel series of URB524 derivatives characterized by the presence of substituents on the "proximal" phenyl ring: the phenyl ring directly bound to the carbamate oxygen atom. The activity of such compounds could help our understanding of the mechanism of carbamate inhibitors of FAAH, as the phenylphenoxide ion is presumed to act as a leaving group during the irreversible inhibition of FAAH mediated by URB524 and related carbamates.[14]

We began the investigation with the insertion of either a nitro or an amino group—groups that are endowed with opposite electronic properties—at the *ortho* and *para* positions of the proximal phenyl ring of URB524 (compounds **2a**–**d**, Table 1). Subsequently we focused our attention on the *para* position, where we introduced either the hydrophilic, electron-withdrawing carboxamido group to produce **2e** or the lipophilic, electron-donating methyl group to give **2f**. These two compounds provided independent variation of the lipophilic and electronic properties of the substituents while maintaining limited steric hindrance. This initial set was expanded to other substituents (compounds **2g**–**j**) with the aim of further testing the effect exerted by hydrogen bonding and steric hindrance. We also prepared two compounds in which the distal phenyl ring of URB524 was significantly modified. Compound **2k** is similar in size and shape to the parent compound, but has an electron-donating pyrrole ring fused onto the proximal phenyl nucleus; **2l** has a lipophilic moiety linked to the proximal phenyl ring through an oxygen atom.

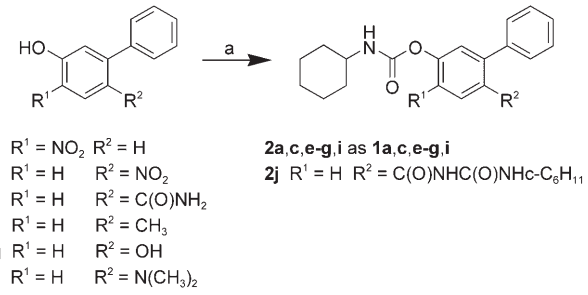
Table 1. Inhibitory potency of tested compounds on FAAH activity.



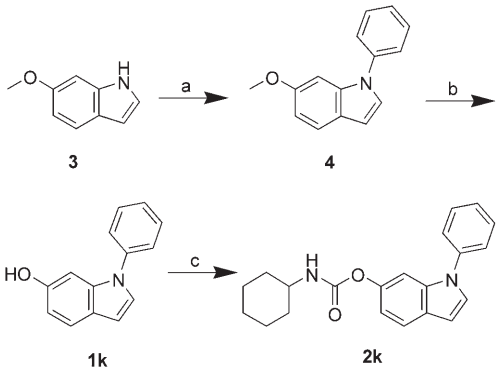
Compd	R^1	R^2	IC_{50} [nM] ± SEM
URB524	H	H	63 ± 9
2a	NO_2	H	> 30 000
2b	NH_2	H	4830 ± 474
2c	H	NO_2	> 30 000
2d	H	NH_2	52 ± 4
2e	H	$C(O)NH_2$	252 ± 48
2f	H	CH_3	176 ± 26
2g	H	OH	45 ± 17
2h	H	CH_2OH	46 ± 5
2i	H	$N(CH_3)_2$	1592 ± 76
2j	H	$C(O)NHC(O)NH$–*c*-C_6H_{11}	2739 ± 940
2k			1029 ± 337
2l			2737 ± 524

Results and Discussion

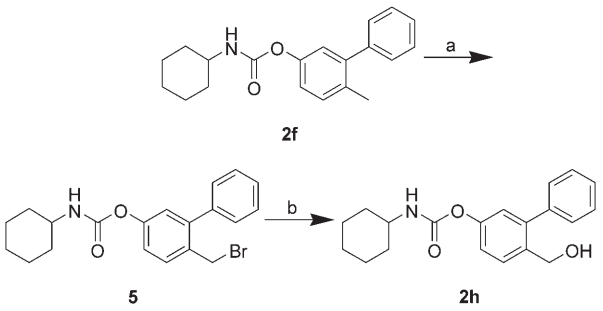
The cyclohexylcarbamic acid aryl esters **2a**,**c**,**e**–**g**,**i**–**l** were obtained by the addition of cyclohexylisocyanate to phenylphenols **1a**,**c**,**e**–**g**,**i** (Scheme 1), indolyl-6-ol **1k** (Scheme 2), and biphenyloxyphenol **1l**. Compound **2h** was obtained through the bromide radical substitution of **2f** with *N*-bromosuccinimide (NBS) and 2,2′-azobisisobutyronitrile (AIBN),[15] and the subsequent hydroxylation of derivative **5** (Scheme 3). Compounds **2b**,**d** were derived from hydrogenation of the nitro groups of **2a**,**c**. Biphenyl-3-ols substituted at the 4 or 6 positions **1a**,**c**,**e** and 3′-phenoxybiphenyl-3-ol (**1l**) were obtained by hydrolysis of the corresponding 3-methoxyaryls with boron tribromide (for compounds **1a**,**e**), lithium chloride[16] (for compound **1c**), or hydiodric acid (for compound **1l**). The 3-methoxyaryl compounds were prepared by Suzuki cross-coupling of phenylboronic acid (**7**) and compounds **6e**,**l**[17] (Scheme 4), or by nitration of 3-methoxybiphenyl[18] in the case of compounds **8a**,**c** (for which the compound numbering scheme follows analogously that of compounds **2** in Table 1). Compound **1f** was synthesized by the reaction of **7** and 3-bromo-4-methylphenol (**6f**)[19] (Scheme 4). Compound **1i** was obtained by dimethylation of



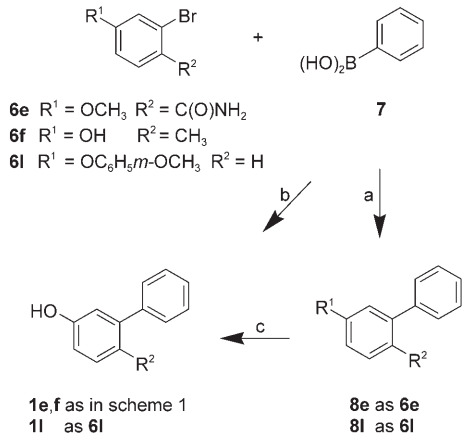
Scheme 1. Reagents and conditions: a) *c*-$C_6H_{11}NCO$, Et_3N (for **1e**–**g**,**i**–**l**) or pyridine (for **1a**,**c**), toluene, reflux, 1–20 h.



Scheme 2. Reagents and conditions: a) C_6H_5I, K_2CO_3, CuI, ZnO, 1-methyl-2-pyrrolidinone, 155 °C, 6 h; b) BBr_3 in CH_2Cl_2, 25 °C, 1 h; c) *c*-$C_6H_{11}NCO$, Et_3N, toluene, reflux, 8 h.



Scheme 3. Reagents and conditions: a) NBS, AIBN, CCl_4, reflux, 18 h; b) $CH_3C(O)CH_3$, $AgNO_3$, 25 °C, 48 h.



Scheme 4. Reagents and conditions: a,b) $Pd(PPh_3)_4$, toluene, Na_2CO_3/H_2O, EtOH, reflux, 0.5–8 h; c) BBr_3, 25 °C, 20 h (for **1e**) or HI, reflux, 3 h (for **1l**).

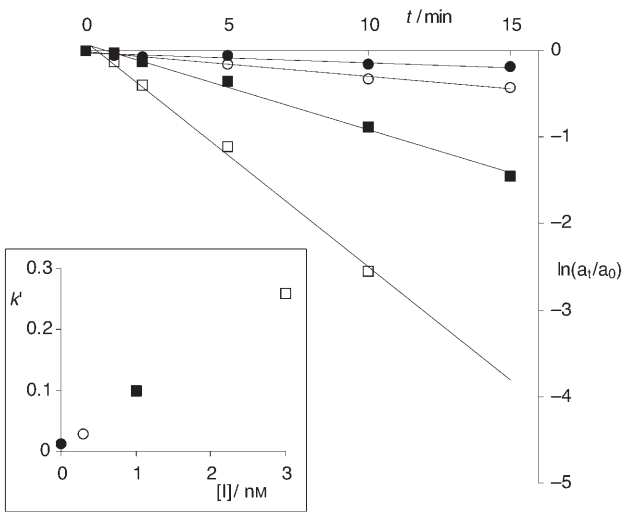


Figure 1. Time course of FAAH inhibition by **2g**. Points represent average values of three independent measurements (control: ●, 0.3 nM: ○, 1.0 nM: ■, 3.0 nM: □) expressed in terms of residual FAAH activity (a_t) over activity at the start time (a_0). Error bars are omitted for clarity, but the uncertainty of individual values was within 10% of the measured inhibition (< 0.1 unit on ln scale). Inset: plot of apparent first-order rate constant (k') over inhibitor concentration.

6-aminobiphenyl-3-ol (**1d**), which was prepared by the hydrogenation of **1c**. Compound **1k** resulted from the hydrolysis (BBr_3) of 1*H*-phenyl-6-methoxyindole (**4**), the synthesis of which involves the formation of 6-methoxyindole (**3**)[20] and subsequent N-arylation by an Ullmann-type reaction[21] (Scheme 2). Compound **6e** was synthesized from the corresponding carboxylic acid, which was obtained[22] by oxidative cleavage of the acetyl derivative.[23]

FAAH activity was measured in rat brain membranes by using [^{3}H]anandamide as a substrate. Half-maximal inhibitory concentrations (IC_{50}) for all compounds are reported in Table 1. The time course of FAAH inhibition was investigated for **2g**, one of the most potent inhibitors, by pre-incubation of rat membranes with the inhibitor. As shown in Figure 1, the residual activity (expressed logarithmically) varies linearly with time. This is consistent with irreversible inhibition brought about by the reaction of the carbamic group with Ser241 of FAAH, as has been postulated previously for this compound class,[12,14] following the scheme:

$$E{-}OH + X{-}Y \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} [E{-}OH \cdot X{-}Y] \xrightarrow{k_{+2}} E{-}OX + HY \qquad (1)$$

in which X–Y is the cleavable carbamic acid derivative and [E–OH·X–Y] represents the noncovalent enzyme–inhibitor complex. Seemingly in contrast with specific irreversible inhibition, which would be expected lead to a hyperbolic, saturable dependence of the apparent first-order rate constant (k') on the inhibitor concentration ([I]),[24] in our case k' is proportional to [I] (Figure 1, inset). We assumed that this linearity, similar to that previously reported for other carbamate inhibitors of esterases,[25,26] may depend on the fact that k_{+2} is not negligible with respect to k_{-1}. Mechanistically, this can be interpreted as a very efficient coupling between the inhibitor fitting into the receptor cavity and its subsequent cleavage. Alternatively, the linear dependence observed between k' and [I] may depend on the fact that the binding site is far from saturation with an inhibitor concentration of 3 nM.

As expected from time-dependent inhibition, the IC_{50} value also changed with pre-incubation. In the case of **2g** the IC_{50} value changed from 45 to 0.43 nM with a pre-incubation time of 15 min; under the same conditions, it changed from 4830 to 227 nM in the case of the weaker inhibitor **2b**.

The first set of compounds (**2a**–**d**) revealed that the insertion of a nitro group at either the *ortho* or *para* position of the proximal phenyl ring of URB524 is detrimental for FAAH inhibition. The low activities observed for these compounds are apparently inconsistent with the hypothesis that stabilization of the phenylphenoxide ion should increase the potency of inhibition. However, this could be explained by the fact that contrary to other compounds of this series, both **2a** and **2c** were subject to a rapid chemical hydrolysis at pH 7.4, with half lives shorter than 2 min (data not shown).

The striking difference in potency between the two amino derivatives **2b** and **2d** provided the first hint that reactivity alone cannot explain the inhibitory potency of this compound class and indicated that the *para* position is the more promising site for further SAR investigation. Therefore, two additional

para-substituted analogues of URB524, **2e** and **2f**, were prepared and tested. Both compounds were slightly less potent than the parent molecule. The electron-withdrawing carbamoyl group of **2e** had a more unfavorable impact on its potency than did the electron-donating methyl group of **2f**. Compounds **2k** and **2l** are characterized by an *O*-carbamoyl substituent different from the *meta*-biphenyl moiety of URB524. The structure of compound **2k** is sterically similar to that of URB524 and is characterized by an electron-rich indole nucleus, whereas in **2l** a lipophilic biphenyl moiety is linked to an *O*-phenyl ring. Both compounds were much less potent than URB524, which probably indicates that lipophilic moieties may not favor a strong interaction between the inhibitor and the binding site of the enzyme. This is similar to what we have previously observed for derivatives of URB524 substituted at the distal phenyl ring.[12]

We therefore turned our attention back to the *para* position. As we had observed that the amino derivative **2d** was the only compound that maintained the potency of URB524, we introduced small, electron-donating substituents in compounds **2g** and **2i**; we also thought it useful to test compound **2h**, a higher homologue of **2g**. Good inhibitory potencies, similar or slightly better than that of URB524, were obtained with small substituents such as hydroxy (in **2g**) and hydroxymethyl (in **2h**) groups, whereas both the dimethylamino derivative **2i** and the bulky derivative **2j** showed a marked decrease in potency. Thus, only small hydrogen-bond donor substituents appear to be tolerated at the *para* position.

To test our initial hypothesis that the inhibitory potency is influenced by stabilization of the leaving group, we sought a quantitative relation between IC_{50} values and the electronic effects of the substituent on the phenyl ring *O*-linked to the carbamate moiety. Although IC_{50} values depend on experimental conditions, they provide a quantitative scale of inhibitory potency. For irreversible inhibitors, this is expected to be the combined result of a recognition step (affinity of the inhibitor for the binding site) and of the propensity of the carbamate group to react with the Ser241 nucleophile. In different cases, this reactivity has been shown to be modulated by the electronic effects of substituents at the phenol moiety.[27] These effects were parameterized by calculating two orbital energies with a quantum mechanical DFT method (Experimental Section).[28] For the *para*-substituted derivatives **2c**–**i** and URB524, a good correlation was observed between the energy of the highest occupied molecular orbital (HOMO, reported in Table 2 with other descriptors employed in QSAR) and the Hammett σ_p [Eq. (2)], indicating that its value is a reliable descriptor of substituent electronic effect.

$$\sigma_p = -30.854(\pm 2.678)\mathrm{HOMO} - 7.064(\pm 0.605)$$
$$n = 8;\ r^2 = 0.957;\ s = 0.118;\ F = 132.7;\ q^2 = 0.919; \quad (2)$$
$$SDEP = 0.140$$

The same calculation was run for all the tested compounds, but no clear correlation between HOMO energies and pIC_{50} values could be observed.

Table 2. Data used in QSAR analysis.

Compd	pIC_{50}	HOMO[a]	LUMO[a]	Vol [Å^3]	HD	π[b]	σ_p[b]
URB524	7.20	−0.23086	−0.03820	277.96	0	0	0
2a	< 4.50	−0.25364	−0.10254	305.253			
2b	5.32	−0.20263	−0.02913	283.169			
2c	< 4.50	−0.25104	−0.09288	296.068	0	−0.28	0.78
2d	7.28	−0.20338	−0.03129	278.640	1	−1.23	−0.66
2e	6.60	−0.23669	−0.04229	297.291	1	−1.49	0.36
2f	6.75	−0.22929	−0.03092	279.805	0	0.56	−0.17
2g	7.35	−0.21834	−0.03361	270.619	1	−0.67	−0.37
2h	7.34	−0.23114	−0.03150	297.373	1	−1.03	0.00
2i	5.80	−0.20213	−0.03091	333.703	0	0.18	−0.83
2j	5.56	−0.24569	−0.05458	412.642	1		
2k	5.99	−0.20718	−0.02998	300.177	0		
2l	5.56	−0.22400	−0.04259	363.645	0		

[a] Orbital energies, calculated as explained in the Experimental Section, are in Hartree. [b] Values of π and σ_p are taken from reference [42].

Excluding derivatives **2a**–**c** from the training set (as **2a** and **2b** are *ortho* substituted and **2c** is chemically instable), only a tentative QSAR model could be obtained by multiple regression analysis (MRA) of the data reported in Table 2. This is described by Equation (3), in which "HD" is an indicator variable set to 1 for compounds that have a group capable of acting as a hydrogen-bond donor and "Vol" is the van der Waals volume.

$$pIC_{50} = 0.570(\pm 0.205)\mathrm{HD} - 0.014(\pm 0.002)\mathrm{Vol} + 10.536(\pm 0.748)$$
$$n = 10;\ r^2 = 0.856;\ s = 0.324;\ F = 20.8;\ q^2 = 0.716; \quad (3)$$
$$SDEP = 0.381$$

This QSAR model supports the idea that for the present series of carbamate inhibitors, recognition events have an overwhelming effect in the determination of inhibitory potency. The slight increase in potency in the case of **2g** is not entirely attributable to the electron-donating properties of its substituent. In fact, the inactive compounds **2i** and **2k** both have HOMO energy intermediate values between those of the dimethylamino (compound **2i**) and hydroxy (compound **2g**) derivatives, and shapes very similar to that of the biphenyl moiety of URB524.

To further validate our assumptions, the compounds of the present series were docked into the catalytic site of FAAH, the crystallographic coordinates of which have been reported.[29] The docking solutions, similar to those previously reported for this class of compounds,[12] suggest that the reason for the limited steric tolerance at the *para* position of the proximal phenyl ring is the result of the presence of bulky amino acid side chains in the surrounding region. These amino acids form the saddle point at the bifurcation of the fatty acid binding cavity and are important in the definition of the size and shape of the active site. In fact, it has been reported that mutation of one of these amino acids, Ile491 to Ala, strongly decreases the binding affinity of *p*-nitrophenyl fatty acid amides with medium-length chains, whereas the mutation has no effect for longer chains.[30] Moreover, molecular dynamics (MD)

simulation for the complex of FAAH and the hydroxymethyl derivative **2h**, which represents the end point of the recognition step, showed that the hydroxy group can establish a polar interaction with the carbonyl group of Gly239, which assists in the arrangement of the carbamic group to a position favorable for nucleophilic attack by Ser241. In fact, during MD simulation (1 ns), compound **2h** remained stably accommodated within the FAAH active site, as can be observed from the distance between its carbonyl carbon atom and the hydroxy group oxygen atom of the catalytic Ser241, as shown in Figure 2; the hydrogen bond between the hydroxymethyl substituent and Gly239 was also stable.

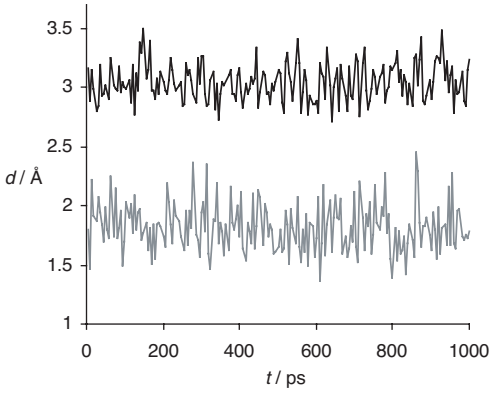


Figure 2. MD simulation (1 ns) performed on the Michaelis complex: distance as a function of time between the carbonyl carbon atom of compound **2h** and the oxygen atom of catalytic Ser241 of FAAH (black line), and between the polar hydrogen atom of the hydroxymethyl substituent in **2h** and the carbonyl oxygen atom of Gly239 (gray line).

In the case of *p*-amino and *p*-hydroxy derivatives **2d** and **2g** it was also possible to obtain docked conformations that maintained the interaction with Gly239 during MD simulations, but only with the interposition of a water molecule. Models of the tetrahedral intermediates were also built (Figure 3A), and MD simulations on these models afforded results similar to those obtained for the Michaelis complexes. The mean distance between the carbonyl oxygen atom of Gly239 and the hydrogen atom of the hydroxymethyl substituent of compound **2h** was 1.90 ± 0.16 Å, calculated from 200 snapshots collected over a period of 1 ns in MD simulation.

An alternative binding mode has been proposed for cyclohexylcarbamic acid biphenyl-3-yl esters on the basis of docking studies performed with URB597 and from mass spectrometric analysis.[12,14] The biphenyl moiety can be accommodated in the funnel pointing toward the cytosolic outlet, with the *N*-cyclohexyl ring directed toward the membrane. Compounds with small substituents at the *para* position of the proximal phenyl ring could be docked in this alternative orientation as well. Furthermore, additional polar interactions with the protein backbone could be undertaken by small polar substituents (in compounds **2d**, **2g**, and **2h**), which can accept a hydrogen bond from the NH group of Cys269 with (**2d** and **2g**) or without (**2h**) the interposition of a water molecule. This interaction

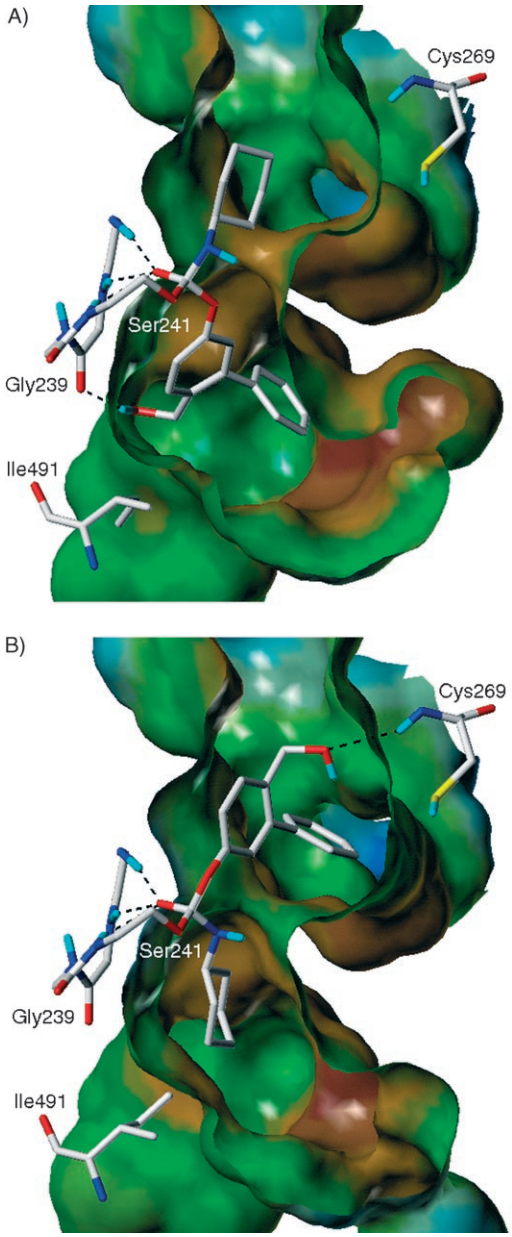


Figure 3. Energy-minimized tetrahedral intermediate of **2h** modeled into the FAAH binding site in the two alternate orientations discussed in the text. The amino acid residues that interact with the *p*-hydroxymethyl group are indicated, and hydrogen bonds are shown (-----). The surface of the enzyme channel is colored according to lipophilicity (brown: high lipophilicity, blue: high hydrophilicity).

was also observed for the tetrahedral intermediate represented in Figure 3B.

Another MD simulation, performed on the Michaelis complex for **2h**, provided evidence that the distance between the nucleophilic oxygen atom of Ser241 and the carbonyl carbon atom of the inhibitor was maintained (mean distance: 3.28 ± 0.16 Å), whereas the interaction between the hydroxymethyl group and the backbone NH of Cys269 was lost during the early phases of the simulation. This resulted from a major rearrangement of the loop at the end of the cytosolic funnel (residues 266–279) that could be strongly affected by boundary

conditions, as this loop is in close contact with a second unit of FAAH observed in the crystal structure.[29] Similar results were also obtained for the corresponding tetrahedral intermediate. Although the role of protein flexibility should be further investigated, the first putative binding mode gives a better explanation for the effect of *para* polar groups, whereas the second mode appears more consistent with the enzyme mechanism. In fact, the second orientation places the biphenyl ester group in close proximity to Ser 217, which belongs to the catalytic triad and is thought to be responsible for the protonation of the putative leaving group: the biphenyl-3-ol anion. These observations suggest that the putative formation of a hydrogen bond between the polar substituents and the binding site can be attributed either to an enhancement of the recognition step between the enzyme and the inhibitor, or to an additional peripheral stabilization of the tetrahedral intermediate, or to both of these effects. Moreover, the molecular models explain the positive coefficient for the HD indicator variable in Equation (3), and suggest that substituents of limited size could overcome the effect of their steric hindrance through a polar interaction.

Conclusions

Based on the hypothesis that the inhibition mechanism of the compounds reported herein involves a carbamoylation of the catalytic serine residue of FAAH, we initially expected an increase in potency from modulation of the electronic features at the proximal phenyl ring of URB524, particularly for electron-withdrawing substituents. This was not the case, however. Rather, the recognition step of the inhibition process, based on stereoelectronic complementarity between the inhibitor and the binding cavity of the enzyme, seems to play a primary role as previously observed for the distal phenyl-ring-substituted analogues of URB524.[12] Furthermore, such a hypothesis may explain why aryl carbamic acid esters are more potent than their alkyl counterparts.[11] The lack of correlation between electronic substituent effects and inhibitory potency may be explained by the chemical instability of some of these carbamate compounds; this is evident for the two nitro derivatives **2a**,**c**. Whereas further investigation is necessary to clarify the exact mechanism of this class of inhibitors, and the application of quantum mechanics can be useful in the elucidation of the relationships between potency and reactivity,[31] the SAR indications reported herein can be applied to the design of new compounds with improved pharmacological properties.

Experimental Section

Chemistry. All chemicals were purchased from Aldrich in the highest quality commercially available. Solvents used were RP grade, unless otherwise indicated. Chromatographic separations were performed on silica gel columns by flash chromatography (Kieselgel 60, 0.040–0.063 mm, Merck). TLC analyses were performed on precoated silica gel on glass sheets (Kieselgel 60 F_{254}, Merck). Melting point data were determined with a Büchi SMP-510 capillary melting point apparatus and are uncorrected. 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 an AVANCE Bruker 200 spectrometer; experiments for the structural determination of **2g** were recorded on an AVANCE Bruker 500 spectrometer; chemical shifts were measured by using the central peak of the solvent. IR spectra were obtained either with a Shimadzu FT-8300, or a Nicolet Atavar spectrometer. Elemental analyses were performed on a Carlo Erba analyzer.

Synthesis of cyclohexylcarbamic acid aryl esters (**2a**,**c**,**e**–**g**,**i**–**l**): Et_3N (0.012 g, 0.017 mL, 0.12 mmol) (or pyridine (0.004 g, 0.003 mL, 0.003 mmol) in the case of **2a**,**c**) and *c*-$C_6H_{11}NCO$ (0.275 g, 0.28 mL, 2.2 mmol) were added to a stirred solution of the appropriate aryl alcohol **1a**,**c**,**e**–**g**,**i**–**l** (2 mmol) in toluene (12 mL). The reactants were kept at reflux for 14 h (**2l**, 1 h; **2k**, 5 h). For **2k** and **2f**, a further amount of *c*-$C_6H_{11}NCO$ (0.138 g, 0.14 mL, 1.1 mmol for **2k**; 0.275 g, 0.28 mL, 2.2 mmol for **2f**) was added, and the mixture was allowed to react again (**2k**, 3 h; **2f**, 6 h). The mixture was then cooled and concentrated. Purification of the residue by column chromatography (cyclohexane/EtOAc 85:15 for **2f**,**l**; 1:1 for **2g**,**j**; 4:1 for **2c**; 9:1 for **2a**,**k**; EtOAc for **2e**) and recrystallization gave **2a**,**c**,**e**–**g**,**i**–**l**.

Cyclohexylcarbamic acid 4-nitrobiphenyl-3-yl ester (**2a**): off-white solid; yield: 37% (0.255 g); mp: 134–136 °C (EtOH); MS (EI): *m/z* 215 (100); ^{1}H NMR ($CDCl_3$): $\delta = 1.26$–2.18 (m, 10H), 3.60 (m, 1H), 5.19 (br d, 1H), 7.46–7.65 (m, 7H), 8.15 ppm (m, 1H); IR (Nujol): $\tilde{\nu} =$ 3323, 1716 cm^{-1}; Anal. calcd for $C_{19}H_{20}N_2O_4$ (340.48): C 67.05, H 5.92, N 8.23, found: C 67.25, H 5.96, N 8.29.

Cyclohexylcarbamic acid 6-nitrobiphenyl-3-yl ester (**2c**): off-white needles; yield: 85% (0.581 g); mp: 132–134 °C (EtOH); MS (EI): *m/z* 215 [M^+], 198 (100); ^{1}H NMR ($CDCl_3$): $\delta = 1.19$–2.03 (m, 10H), 3.56 (m, 1H), 4.97 (br d, 1H), 7.22–7.40 (m, 7H), 7.91 ppm (d, 1H); IR (Nujol): $\tilde{\nu} = 3321$, 1709 cm^{-1}; Anal. calcd for $C_{19}H_{20}N_2O_4$ (340.48): C 67.05, H 5.92, N 8.23, found: C 67.21, H 6.01, N 8.47.

Cyclohexylcarbamic acid 6-carbamoylbiphenyl-3-yl ester (**2e**): white needles; yield: 6% (0.041 g); mp: 209–211 °C (EtOAc); MS (EI): *m/z* 338 [M^+], 197 (100); ^{1}H NMR ($CDCl_3$): $\delta = 1.18$–2.07 (m, 10H), 3.50 (m, 1H), 4.99 (br d, 1H), 5.23 (br s, 1H), 5.57 (br s, 1H), 7.15–7.43 (m, 7H), 7.81 ppm (d, 1H); IR (Nujol): $\tilde{\nu} = 3332$, 1705 cm^{-1}; Anal. calcd for $C_{20}H_{22}N_2O_3$ (338.41): C 70.99, H 6.55, N 8.28, found: C 71.07, H 6.69, N 8.33.

Cyclohexylcarbamic acid 6-methylbiphenyl-3-yl ester (**2f**): white scales; yield: 90% (0.554 g); mp: 153–155 °C (EtOH); MS (EI): *m/z* 310 [M^+], 184 (100); ^{1}H NMR ($CDCl_3$): $\delta = 1.22$–2.06 (m, 10H), 2.25 (s, 3H), 3.55 (m, 1H), 4.90 (br d, 1H), 7.01–7.36 ppm (m, 8H); IR (Nujol): $\tilde{\nu} = 3291$, 1740, 1702 cm^{-1}; Anal. calcd for $C_{20}H_{23}NO_2$ (309.41): C 77.64, H 7.49, N 4.53, found: C 77.82, H 7.51, N 4.66.

Cyclohexylcarbamic acid 6-hydroxybiphenyl-3-yl ester (**2g**):[32] white crystals; yield: 35% (0.226 g); mp: 118–119 °C (CH_2Cl_2/petroleum ether); MS (EI): *m/z* 311 [M^+], 186 (100); ^{1}H NMR ($CDCl_3$): $\delta =$ 1.17–2.00 (m, 10H), 3.55 (m, 1H), 4.90 (br d, 1H), 5.28 (s, 1H), 6.86 (d, 1H), 6.96 (q, 1H), 7.00 (q, 1H), 7.34–7.47 ppm (m, 5H); IR (Nujol): $\tilde{\nu} = 3342$, 1704 cm^{-1}; Anal. calcd for $C_{19}H_{21}NO_3 \cdot 0.1\,CH_2Cl_2$ (319.87): C 71.72, H 6.68, N 4.38, found: C 71.45, H 6.59, N 4.56.

Cyclohexylcarbamic acid 6-dimethylaminobiphenyl-3-yl ester (**2i**): white needles; yield: 98% (0.661 g); mp: 153–154 °C (cyclohexane); MS (EI): *m/z* 338 [M^+], 213 (100); ^{1}H NMR ($CDCl_3$): $\delta = 1.19$–2.03 (m, 10H), 2.58 (s, 6H), 3.55 (m, 1H), 4.89 (s, 1H), 7.01–7.53 ppm (m, 8H); IR (Nujol): $\tilde{\nu} = 3310$, 1710 cm^{-1}; Anal. calcd for $C_{21}H_{26}N_2O_2$ (338.45): C 74.53, H 7.74, N 8.28, found: C 74.97, H 7.81, N 8.40.

Cyclohexylcarbamic acid 6-(3-cyclohexylureidocarbonyl)biphenyl-3-yl ester (**2j**): isolated as a side product of **2e**; white crystals; yield: 45% (0.416 g); mp: 165–169 °C (EtOAc); MS (EI): *m/z* 197 (100); ^{1}H NMR ($CDCl_3$): δ = 1.16–2.05 (m, 20H), 3.60 (m, 2H), 5.06 (br d, 1H), 7.20–7.42 (m, 8H), 7.70 (d, 1H), 8.19 ppm (dd, 1H); IR (Nujol): $\tilde{\nu}$ = 3307, 1697 cm^{-1}; Anal. calcd for $C_{27}H_{33}N_3O_4$ (463.58): C 69.96, H 7.18, N 9.06, found: C 69.99, H 7.30, N 8.92.

Cyclohexylcarbamic acid 1-phenyl-1*H*-indol-2-yl ester (**2k**): white solid; yield: 68% (0.453 g); mp: 140 °C (EtOH); MS (EI): *m/z* 334 [M^+], 91 (100); ^{1}H NMR ($CDCl_3$): δ = 1.17–2.04 (m, 10H), 3.59 (m, 1H), 4.90 (br d, 1H), 6.66 (m, 1H), 6.95 (dd, 1H, *J* = 8.3), 7.33 (m, 3H), 7.50 (m, 4H), 7.62 ppm (d, 1H, *J* = 8.6); IR (KBr): $\tilde{\nu}$ = 3288, 1706 cm^{-1}; Anal. calcd for $C_{21}H_{22}N_2O_2$ (334.42): C 75.48, H 6.63, N 8.38, found: C 75.00, H 6.31, N 8.23.

Cyclohexylcarbamic acid 3-(biphenyl-3-yloxy)phenyl ester (**2l**): white crystals; yield: 87% (0.673 g); mp: 101–102 °C (EtOH); MS (EI): *m/z* 387 [M^+], 262 (100); ^{1}H NMR ($CDCl_3$): δ = 1.17–2.03 (m, 10H), 3.57 (m, 1H), 4.88 (br d, 1H), 6.84–7.60 ppm (m, 13H); IR (KBr): $\tilde{\nu}$ = 3316, 1739, 1708 cm^{-1}; Anal. calcd for $C_{25}H_{25}NO_3$ (387.48): C 77.49, H 6.50, N 3.61, found: C 77.47, H 6.13, N 3.21.

Synthesis of cyclohexylcarbamic acid 6-hydroxymethylbiphenyl-3-yl ester (**2h**): NBS (0.274 g, 1.54 mmol) and 2,2′-azobisisobutyronitrile (0.014 g, 0.086 mmol) were added to a stirred solution of **2f** (0.464 g, 1.5 mmol) in CCl_4 (20 mL). The mixture was kept at reflux for 18 h then cooled, extracted with CH_2Cl_2, washed with H_2O, dried (Na_2SO_4), and concentrated. Purification of the residue by column chromatography (cyclohexane/EtOAc 9:1) afforded pure cyclohexylcarbamic acid 6-bromomethylbiphenyl-3-yl ester (**5**) [^{1}H NMR ($CDCl_3$): δ = 1.18–2.04 (m, 10H), 3.57 (m, 1H), 4.44 (s, 2H), 4.93 (br d, 1H), 7.01–7.54 ppm (m, 10H)], which was dissolved in $CH_3C(O)CH_3$ (20 mL), added to an aqueous solution of $AgNO_3$ (0.2 N, 1.75 mL) and stirred at room temperature for 48 h. The mixture was filtered, concentrated, and dissolved (EtOAc). The organic phase was washed with a saturated aqueous solution of $NaHCO_3$ and brine, dried (Na_2SO_4), and concentrated. Purification of the residue by column chromatography (cyclohexane/EtOAc 8:2) and recrystallization gave **2h** as a white solid. Yield: 30% (0.149 g); mp: 141–142 °C (Et_2O/petroleum ether); MS (EI): *m/z* 200 (100); ^{1}H NMR ($CDCl_3$): δ = 0.86–2.07 (m, 11H), 3.57 (m, 1H), 4.58 (s, 2H), 4.99 (br d, 1H), 7.06–7.55 ppm (m, 8H). IR (Nujol): $\tilde{\nu}$ = 3439, 1731 cm^{-1}; Anal. calcd for $C_{20}H_{23}NO_3 \cdot 0.1\,c\text{-}C_6H_{12}$ (333.82): C 74.12, H 7.31, N 4.20, found: C 74.40, H 7.00, N 3.88.

Synthesis of cyclohexylcarbamic acid aminobiphenyl-3-yl esters (**2b,d**)**:** Pd/C (10%, 0.015 g) was added to a stirred suspension of the appropriate carbamic acid nitrobiphenyl ester **2a,c** (0.170 g, 0.5 mmol) in EtOH (15 mL). The mixture was hydrogenated (3 atm, 50–60 °C) for 14 h, cooled, filtered on celite, and concentrated. Purification of the residue by column chromatography (cyclohexane/EtOAc 1:1 for **2b**; 7:3 for **2d**) gave **2b,d** as solids.

Cyclohexylcarbamic acid 4-aminobiphenyl-3-yl ester (**2b**): white solid; yield: 36% (0.056 g); mp: 186–188 °C (EtOH); MS (EI): *m/z* 310 [M^+], 185 (100); ^{1}H NMR ($CDCl_3$): δ = 1.11–2.03 (m, 10H), 3.56 (br s, 1H), 3.75 (br s, 2H), 4.89 (br d, 1H), 6.85–7.94 ppm (m, 8H); IR (Nujol): $\tilde{\nu}$ = 3441, 3354, 3300, 1725, 1692, 1643 cm^{-1}; Anal. calcd for $C_{19}H_{22}N_2O_2$ (310.40): C 73.52, H 7.14, N 9.03, found: C 73.59, H 7.26, N 8.97.

Cyclohexylcarbamic acid 6-aminobiphenyl-3-yl ester (**2d**): sand-colored crystals; yield: 83% (0.131 g); mp: 123–125 °C (EtOH); MS (EI): *m/z* 310 [M^+], 185 (100); ^{1}H NMR ($CDCl_3$): δ = 1.09–2.02 (m, 10H), 3.55 (m, 3H), 4.97 (br d, 1H), 6.69–7.37 ppm (m, 8H); IR (Nujol): $\tilde{\nu}$ = 3333, 1717 cm^{-1}; Anal. calcd for $C_{19}H_{22}N_2O_2 \cdot 0.5\,H_2O$ (319.40): C 71.45, H 7.26, N 8.77, found: C 71.64, H 7.03, N 8.60.

Synthesis of 4- or 6-substituted biphenyl-3-ols (**1a,e**): A solution of the appropriate methoxybenzene **8a,e** (2.5 mmol) in dry CH_2Cl_2 (30 mL) under N_2 atmosphere was added to a stirred, cooled (0 °C), solution of BBr_3 (1 M, 6.5 mL) in CH_2Cl_2. The mixture was stirred at room temperature for the appropriate time (1 h for **1a**; 20 h for **1e**) then quenched with Na_2CO_3 (2 N) and extracted with EtOAc. The combined organic phases were dried (Na_2SO_4) and concentrated. Purification of the residue by column chromatography (cyclohexane/CH_2Cl_2 7:3 for **1a**, EtOAc for **1e**) gave **1a,e** as solids.

4-Nitrobiphenyl-3-ol (**1a**):[33] yellow crystals; yield: 83% (0.445 g); mp: 101–103 °C (EtOH) [lit.: 104–105 °C (EtOH)];[33] MS (EI): *m/z* 215 [M^+], 185, 168, 157, 139 (100), 128, 115, 77, 69, 63; ^{1}H NMR ($CDCl_3$): δ = 7.21–7.66 (m, 7H), 8.18 (d, 1H), 10.72 ppm (s, 1H); IR (Nujol): $\tilde{\nu}$ = 3207 cm^{-1}.

5-Hydroxybiphenyl-2-carboxylic acid amide (**1e**): pale brown solid; yield: 98% (0.521 g); mp: 143–145 °C (EtOAc); MS (EI): *m/z* 213 [M^+], 77 (100); ^{1}H NMR ($CDCl_3$): δ = 5.20 (br s, 1H), 5.46 (br s, 1H), 6.85 (m, 2H), 7.36–7.61 ppm (m, 7H); IR (KBr): $\tilde{\nu}$ = 3440–2924, 1643 cm^{-1}.

Synthesis of 6-nitrobiphenyl-3-ol (**1c**):[33] LiCl (1.90 g, 45 mmol) was added to a stirred solution of **8c** (3.437 g, 15 mmol) in DMF (35 mL). After keeping the reactants at reflux for 24 h, a further amount of LiCl (0.728 g, 17 mmol) was added, and the mixture was allowed to react for another 12 h. The mixture was then cooled, added of NaOH (10%), extracted with Et_2O, acidified with HCl (37%), and extracted again with Et_2O. The combined organic layers were dried (Na_2SO_4) and evaporated. Purification of the residue by column chromatography (cyclohexane/EtOAc 7:3) and recrystallization gave **1c** as a yellow solid. Yield 90% (2.907 g); MS (EI): *m/z* 215 [M^+], 198, 186, 170, 159, 139, 131, 128, 115, 84, 77, 69, 63, 56 (100); ^{1}H NMR ($CDCl_3$): δ = 5.80 (s, 1H), 6.86 (m, 2H), 7.36 (m, 2H), 7.44 (m, 3H), 7.94 ppm (d, 1H).

Synthesis of 3′-phenoxybiphenyl-3-ol (**1l**): **8l** (0.691 g, 2.5 mmol) and HI (57%, 3.2 mL) were held at reflux under vigorous stirring for 3 h. The mixture was cooled, diluted with H_2O, neutralized with $NaHCO_3$ (2 N), and extracted with CH_2Cl_2. The combined organic phases were dried (Na_2SO_4) and concentrated. Purification of the residue by column chromatography (cyclohexane/EtOAc 85:15) gave **1l** as a pale yellow oil. Yield: 92% (0.600 g); MS (EI): *m/z* 262 [M^+], 115 (100); ^{1}H NMR ($CDCl_3$): δ = 5.24 (s, 1H), 6.62 (m, 3H), 7.02–7.61 ppm (m, 10H); IR (neat): $\tilde{\nu}$ = 3391 cm^{-1}.

Synthesis of 6-aminobiphenyl-3-ol (**1d**):[34] HCl (37%, 27.5 mL) was added to a stirred suspension of **1c** (2.367 g, 11 mmol) and Fe powder (1.535 g, 27.5 mmol) in EtOH (55 mL). The mixture was kept at reflux for 1.5 h, quenched with a solution of cooled saturated $NaHCO_3$, extracted with EtOAc, and washed with H_2O. The combined organic layers were dried (Na_2SO_4) and concentrated. Purification of the residue by column chromatography (cyclohexane/EtOAc 6:4) and recrystallization from Et_2O gave **1d** as an oil. Yield 25% (0.510 g); MS (EI): *m/z* 185 (100). ^{1}H NMR ($CDCl_3$) according to published data.[32] IR (neat): $\tilde{\nu}$ = 3430 cm^{-1}.

Synthesis of 6-dimethylaminobiphenyl-3-ol (**1i**): HCOOH (12.5 mL) was added to a stirred solution of **1d** (0.463 g, 2.5 mmol) in HCOH (40%, 8.75 mL), and the mixture was kept at reflux overnight, cooled, neutralized with a saturated solution of $NaHCO_3$, and extracted with EtOAc. The combined organic layers were dried (Na_2SO_4) and evaporated. Purification of the residue by column chromatography (cyclohexane/EtOAc 8:2) and recrystallization

gave **1i** as an off-white solid. Yield: 98% (0.520 g); mp: 70–72 °C (Et_2O/petroleum ether); MS (EI): *m/z* 213 (100); ^{1}H NMR ($CDCl_3$): $\delta =$ 2.55 (s, 6H), 6.77–7.57 ppm (m, 9H); IR (KBr): $\tilde{\nu} = 3425, 3039\ cm^{-1}$.

Synthesis of 1-phenyl-1*H*-indol-6-ol (**1k**): A solution of BBr_3 (1 M, 27.5 mL) in CH_2Cl_2 was added to a stirred solution of **4** (5.582 g, 25 mmol) in dry CH_2Cl_2 (330 mL) under Ar atmosphere. The mixture was stirred at room temperature for 1 h, quenched with Na_2CO_3 (2 N), and extracted with EtOAc. The combined organic layers were washed with brine, dried (Na_2SO_4), and concentrated. Purification of the residue by column chromatography (cyclohexane/EtOAc 9:1 → 6:4) gave **1k** as a pale brown oil. Yield: 9% (0.469 g); MS (EI): *m/z* 209 [M^+], 77 (100); the product was not fully characterized because of its instability.

Synthesis of 2-substituted-5-methoxy- or 5-hydroxybiphenyls (**8e**,**1f**), or 3′-methoxy-3-phenoxybiphenyl (**8l**): $Pd(PPh_3)_4$ (0.231 g, 0.2 mmol), a solution of Na_2CO_3 (3.329 g, 31.41 mmol) in H_2O (16 mL), and a solution of phenylboronic acid (**7**) (1.122 g, 10 mmol) in EtOH (14 mL) were added to a stirred solution of the appropriate halobenzene **3e**,**f**,**l** (5 mmol) in toluene (32 mL) under N_2 atmosphere. The mixture was vigorously stirred under reflux for the appropriate time (0.5 h for **8l**, 4 h for **1f**, 8 h for **8e**), cooled, added to H_2O, and extracted with EtOAc. The combined organic layers were dried (Na_2SO_4) and concentrated. Purification of the residue by column chromatography (cyclohexane/EtOAc 200:1 → 100:1 for **8l**, 9:1 for **1f**, EtOAc for **8e**) gave **8e**,**l** as solids and **1f** as an oil.

5-Methoxybiphenyl-2-carboxylic acid amide (**8e**):[35] white crystals; yield: 95% (1.081 g); mp: 178–180 °C (EtOH) [lit.: 182–183 °C];[35] MS (EI): *m/z* 227 [M^+], 139 (100); ^{1}H NMR ($CDCl_3$): $\delta = 3.87$ (s, 3H), 5.14 (br s, 1H), 5.36 (br s, 1H), 6.83 (d, 1H), 6.96 (dd, 1H), 7.44 (s, 5H), 7.85 ppm (d, 1H); IR (KBr): $\tilde{\nu} = 3417, 1644\ cm^{-1}$.

6-Methylbiphenyl-3-ol (**1f**):[36] Amber oil; yield: 90% (0.827 g); MS (EI): *m/z* 184 (100); ^{1}H NMR and IR spectra according to published data.[36]

3-Methoxy-3-phenoxybiphenyl (**8l**):[37] white crystals; yield: 62% (0.859 g); mp: 55–57 °C (cyclohexane) [lit.: 41–42 °C];[37] MS (EI): *m/z* 276 [M^+], 77 (100); ^{1}H NMR ($CDCl_3$): $\delta = 3.80$ (s, 3H), 6.63–6.70 (m, 3H), 7.01 (m, 1H), 7.20–7.59 ppm (m, 9H).

Synthesis of 3-(or 5-)methoxy-2-(or 4-)nitrobiphenyls (**8a**,**c**)**:** A mixture of HNO_3 (65%, 7 mL) and H_2SO_4 (96%, 7 mL) were added to a stirred solution of 3-methoxybiphenyl (12.897 g, 70 mmol) in CH_2Cl_2 (70 mL) at 0 °C over the course of 45 min. The mixture was stirred for 3 h, cooled with ice water, neutralized with $NaHCO_3$, and extracted with CH_2Cl_2. The combined organic layers were dried (Na_2SO_4) and evaporated. Purification of the residue by column chromatography (cyclohexane/CH_2Cl_2 75:25) gave both **8a**,**c**.

3-Methoxy-4-nitrobiphenyl (**8a**):[38] Oil; yield: 10% (1.605 g); MS (EI): *m/z* 229 [M^+] (100), 199, 182, 171, 153, 152, 139, 128, 115, 102, 87, 77, 63 (the fragmentation pattern, substantially different to that of the isomer **8c**, closely parallels that of 2-methyl-6-nitro-3-phenylanisole);[39] ^{1}H NMR ($CDCl_3$): $\delta = 4.04$ (s, 3H), 7.21–7.26 (m, 2H), 7.42–7.64 (m, 5H), 7.97 ppm (d, 1H).

5-Methoxy-2-nitrobiphenyl (**8c**):[40] Oil; yield: 22% (3.530 g); MS (EI): *m/z* 229 [M^+], 212, 200, 184, 173, 168, 158, 152, 140, 139, 130, 128, 115, 102, 89, 87, 77, 69, 63 (the fragmentation pattern closely parallels that of 2-methyl-4-nitro-3-phenylanisole);[39] ^{1}H NMR ($CDCl_3$): $\delta = 3.90$ (s, 3H), 6.87 (d, 1H, $J = 2.8$), 6.95 (dd, 1H, $J = 2.8$ and 8.9), 7.29–7.47 (m, 5H), 7.99 ppm (d, 1H, $J = 8.9$).

Synthesis of 6-methoxy-1-phenyl-1*H*-indole (**4**):[41] A mixture of 6-methoxy-1*H*-indole (**3**) (5.887 g, 40 mmol), C_6H_5I (14.117 g, 69.2 mmol), K_2CO_3 (7.021 g, 50.8 mmol), CuI (1.996 g, 10.48 mmol), ZnO (0.482 g, 5.92 mmol), and 1-methyl-2-pyrrolidinone (8 mL) was heated at 155 °C under stirring for 6 h, cooled (0 °C), and filtered. The filtrate was added to Et_2O, and a dilute solution of NH_4OH. The organic layer was washed with brine, dried (Na_2SO_4), and concentrated. Purification of the residue by column chromatography (cyclohexane/EtOAc 98:2 → 96:4) gave **4** as a yellow oil. Yield: 65% (5.802 g); MS (EI): *m/z* 223 [M^+], 77 (100). ^{1}H NMR ($CDCl_3$): $\delta = 3.83$ (s, 3H), 6.62 (m, 1H), 6.85 (dd, 1H), 7.06 (m, 1H), 7.24 (m, 1H), 7.32 (m, 1H), 7.49–7.59 ppm (m, 5H).

Synthesis of 2-bromo-4-methoxybenzamide (**6e**): $SOCl_2$ (1.428 g, 0.875 mL, 12 mmol) was added to a stirred suspension of 2-bromo-4-methoxybenzoic acid (2.773 g, 12 mmol) in toluene (7.5 mL). The mixture was stirred at 80 °C for 16 h, concentrated, and treated at room temperature with an aqueous solution of ammonia (30%, 3 mL) for 6 h. The product was dissolved in $CH_3C(O)CH_3$ and concentrated. Purification of the residue by column chromatography (EtOAc) gave **6e** as a solid. Yield: 45% (1.244 g); mp: 182–186 °C (EtOH); MS (EI): *m/z* 230 [M^+], 213 (100); ^{1}H NMR ($CDCl_3$): $\delta = 3.85$ (s, 3H), 6.11 (br s, 1H), 6.30 (br s, 1H), 6.91 (d, 1H), 7.14 (s, 1H), 7.74 ppm (d, 1H). IR (KBr): $\tilde{\nu} = 3356, 3177, 1643\ cm^{-1}$.

QSAR and Molecular modeling. Multiple regression analysis (MRA) calculations were performed with an Excel (Microsoft Co., version 97) spreadsheet, employing the built-in statistical functions and automated macro procedures. Substituent constants π and σ_p were taken from the Hansch collection.[42] Other descriptors were calculated as described below. MRA models were calculated for all 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, in which one compound at a time is omitted from the set according to the leave-one-out technique (LOO).[43]

Quantum chemical and volume descriptors: Starting from the conformations docked within the active site of FAAH, molecular models of compounds URB524 and **2a**–**l** were minimized in vacuo to an energy gradient of 0.01 kcal mol^{-1} Å^{-1} by using the MMFF94s[44] force field implemented in Sybyl 6.9.[45] The resulting structures were used as starting input structures for DFT[46] calculations with the B3LYP[47–50] hybrid functional. A double-basis set, augmented with polarization and diffuse functions for non-hydrogen atoms (6–31 + G(d)) was used to optimize the geometry of these molecules; vibrational frequencies were calculated, showing that the resulting structures were minima on the corresponding potential energy surfaces. HOMO and LUMO energies were computed at B3LYP/6-31 + G(d) level of theory and used as quantum chemical descriptors in the QSAR study. DFT calculations were performed with Jaguar 4.2 software.[51] Molecular volume descriptors were calculated for the DFT optimized structures by the Sybyl command *molprop_volume*. The standard van der Waals volume was obtained by setting the probe radius value to 0.

Molecular docking and dynamics: Starting from the coordinates deposited in the Protein Data Bank (PDB code: 1MT5),[29] a functional subunit of the FAAH enzyme was prepared as described[12] to perform docking and MD simulation studies. The inhibitors were docked into the enzyme channel, after which their position and conformation were optimized first by the Sybyl 6.9 *Dock_minimize* procedure, then by energy minimization of the complex with the MMFF94s force field to an energy gradient of 0.1 kcal mol^{-1} Å^{-1}, al-

lowing movements of the residues at maximum of 8 Å from the inhibitor.

The tetrahedral intermediates were built by creating a covalent bond between the oxygen atom of Ser241 and the carbamate carbon atom; the negatively charged oxygen atom was directed toward the oxyanion hole, and the amino group of Lys142 was protonated. Geometry optimization was performed as described for the Michaelis complex.

MD simulations (step size of 1 fs) were performed with the MMFF94 force field implemented in the Macromodel[52] package for 1 ns at 310 K after an equilibration time of 100 ps at the same temperature. During the simulation, only atoms within 8 Å of the inhibitor were allowed to move. Snapshots of the trajectory were saved every 5 ps for subsequent analysis. To check the stability of the Michaelis complexes, the distance between the hydroxy group oxygen atom of Ser241 and the inhibitor carbonyl carbon atom was monitored during MD simulations.

Pharmacology. FAAH inhibition: Membrane fractions were prepared from Wistar rat brain homogenates, and FAAH activity was assayed by using [^{3}H]anandamide (anandamide[ethanolamine-^{3}H], 60 Ci mmol^{-1}, American Radiolabeled Chemicals, St. Louis, USA) as substrate. Membranes (50 μg protein) were incubated for 30 min at 37 °C in Tris buffer (50 mM, pH 7.5, 0.45 mL) containing fatty-acid-free bovine serum albumin (BSA, 0.05% by weight), [^{3}H]anandamide (10000 dpm, 10 μM) and varying concentrations of test compounds. At the end of the incubation period, the reactions were stopped with a mixture of chloroform/methanol and [^{3}H]ethanolamine was measured in a volume of 0.6 mL in aqueous phase by liquid scintillation counting.[11] For some experiments, brain membranes were pre-incubated at 37 °C for various durations with test compounds. Reactions were started by adding [^{3}H]anandamide and were conducted for a further 30 min.

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Keywords: carbamate inhibitors · FAAH · molecular modeling · structure–activity relationships · URB524

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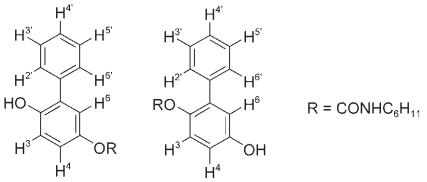
[32] In principle, the reaction leading to **2g** may also have produced the regioisomer *ortho*-carbamoylated biphenyl **9**. Unequivocal structure determination was carried out with two independent NMR experiments:



2g 9

nuclear Overhauser enhancement (nOe) and heteronuclear multiple-bond correlation (HMBC). Concerning nOe, selective irradiation of O*H* is expected to produce an enhancement of signals for H^3, $H^{2'}$, and $H^{6'}$ in isomer **2g**, and for H^4 and H^6 in the case of **9**. In fact, we observed enhancements of 4.1% for $H^{2'}$ and $H^{6'}$ and of 3.3% for H^3, whereas enhancements of the signals for H^4 and H^6 were negligible (data not shown). Concerning HMBC, heteronuclear long-range couplings of O*H* are expected with C_{ipso} and the two C_{ortho} atoms, corresponding to C^2, C^1, and C^3 in isomer **2g**, and to C^5, C^4, and C^6 in isomer **9**. The observed long-range correlations again confirmed structure **2g** (data not shown).

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