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

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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 CB1 and CB2, at least two lipid ligands: N-arachidonylethanolamine (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]

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 fluoro phosphonate,[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 hydroxydocannabinoid 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] compound by introducing a series of substituents at the ortho and para positions. pIC50 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.

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. pIC50 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.

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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.[16]

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 FAAH inhibitors with potency in the low nanomolar concentration range, such as URB5, the pharmacological properties of FAAH inhibitors with potency in the low nanomolar concentration range, such as URB597, the pharmacological properties of which have been described.[16]

We began the investigation with the insertion of either a hydrophilic, electron-donating methyl group to give 2c. 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 2d–g) 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.

### 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 bi-phenyloxypbenol 1l. Compound 2h was obtained through the bromide radical substitution of 2f with N-bromosuccinimide (NBS) and 2,2’-azoisobutyronitrile (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-methoxyaryl with boron tribromide (for compounds 1a,e), lithium chloride[16] (for compound 1c), or hydriodic acid (for compound 1l). The 3-methoxyaryl compounds were prepared by Suzuki cross-coupling of phenylboronic acid (7) and compounds 6e,f,[17] 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

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

| Compd | R' | R'' | IC50 [nM] ± SEM |
|-------|----|-----|----------------|
| URB524 | H | H | 63 ± 9 |
| 2a | NO2 | H | > 30,000 |
| 2b | NH2 | H | 4830 ± 474 |
| 2c | H | NO2 | > 30,000 |
| 2d | H | NH2 | 52 ± 4 |
| 2e | H | C(O)NH | 252 ± 48 |
| 2f | H | CH3 | 176 ± 26 |
| 2g | H | OH | 45 ± 17 |
| 2h | H | CH2OH | 46 ± 5 |
| 2i | H | N(CH3)2 | 1592 ± 76 |
| 2j | H | C(O)NH(C,O)NH–C6H11 | 2739 ± 940 |
| 2k | | | 1029 ± 337 |
| 2l | | | 2737 ± 524 |

Scheme 1. Reagents and conditions: a) C6H5NCO, Et3N (for 1a,c,e–g,i–l) or pyridine (for 1a,c), toluene, reflux, 1–20 h.

Scheme 2. Reagents and conditions: a) C6H5I, K2CO3, CuI, ZnO, 1-methyl-2-pyrrolidinone, 155 °C, 6 h; b) BBri in CH3Cl, 25 °C, 1 h; c) C6H5NCO, Et3N, toluene, reflux, 8 h.
6-aminobiphenyl-3-ol (1d), which was prepared by the hydrogenation of 1c. Compound 1k resulted from the hydrolysis (BBr₃) of 1H-phenyl-6-methoxyindole (4), the synthesis of which involves the formation of 6-methoxyindole (3) and subsequent N-arylation by an Ullmann-type reaction (Scheme 2).

Compound 6e was synthesized from the corresponding carboxylic acid, which was obtained by oxidative cleavage of the acetyl derivative.

FAAH activity was measured in rat brain membranes by using [³H]anandamide as a substrate. Half-maximal inhibitory concentrations (IC₅₀) for all compounds are reported in Table 1. The time course of FAAH inhibition was investigated for 1e of 1k, with respect to [I] (Figure 1, inset). We assumed that this linearity, similar to that previously reported for other carbamate inhibitors of esterases, may depend on the fact that k₋ₓ is not negligible with respect to k₊. 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 IC₅₀ value also changed with pre-incubation. In the case of 2g, the IC₅₀ 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 phenylenoxoide 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.
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-phenoxy 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.\textsuperscript{[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 2l; 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 \textsuperscript{(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 \textit{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\textsubscript{50} values and the electronic effects of the substituent on the phenyl ring O-linked to the carbamate moiety. Although IC\textsubscript{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.\textsuperscript{[27]}

These effects were parameterized by calculating two orbital energies with a quantum mechanical DFT method (Experimental Section).\textsuperscript{[28]} For the \textit{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 \( \alpha_p \) [Eq. (2)], indicating that its value is a reliable descriptor of substituent electronic effect.

\[ \alpha_p = -30.854(\pm 2.678) \times \text{HOMO} - 7.064(\pm 0.605) \]
\[ n = 8; r^2 = 0.957; s = 0.118; F = 132.7; q^2 = 0.919; \]
\[ SDEP = 0.140 \]

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

\begin{table}[h]
\centering
\begin{tabular}{lcccccc}
\hline
Compd & pIC\textsubscript{50} & HOMO & LUMO & Vol [Å\textsuperscript{3}] & HD & \( \pi_b \) \\
\hline
UBR524 & 7.20 & -0.23086 & -0.03820 & 277.96 & 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 & \\
\hline
\end{tabular}
\caption{Data used in QSAR analysis.}
\end{table}

Excluding derivatives 2a–c from the training set (as 2a and 2b are \textit{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.

\[ \text{pIC}_{50} = 0.570(\pm 0.205)\text{HD} - 0.014(\pm 0.002)\text{Vol} + 10.536(\pm 0.748) \]
\[ n = 10; r^2 = 0.856; s = 0.324; F = 20.8; q^2 = 0.716; \]
\[ SDEP = 0.381 \]

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 2f) 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.\textsuperscript{[29]} The docking solutions, similar to those previously reported for this class of compounds,\textsuperscript{[12]} suggest that the reason for the limited steric tolerance at the \textit{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.\textsuperscript{[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.

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. 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 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. 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 Ser217, 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 carbamylation 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. Furthermore, such a hypothesis may explain why aryl carbamic acid esters are more potent than their alkyl counterparts. 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 2ac. 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, 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 60F254, Merck). Melt point data were determined with a Büchi SMP-510 capillary melting point apparatus and are uncorrected. El MS spectra (70 eV) were recorded with a Fisons Trio 1000 spectrometer; only molecular ions ([M+H]+) and base peaks are given. 1H 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 (2acegi). Et3N (0.012 g, 0.017 mL, 0.12 mmol) or pyridine (0.004 g, 0.003 mL, 0.003 mmol) in the case of 2ac and c-C6H14NCO (0.275 g, 0.28 mmol, 2.2 mmol) were added to a stirred solution of the appropriate aryl alcohol 1acegi (2 mmol) in toluene (12 mL). The reagents were kept at reflux for 14 h (2i, 1 h; 2k, 5 h). For 2k and 2f, a further amount of c-C6H14NCO (0.138 g, 0.14 mL, 1.1 mmol for 2k; 0.275 g, 0.28 mmol, 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 2fi; 1:1 for 2gj; 4:1 for 2c; 9:1 for 2ak; EtOAc for 2e) and recrystallization gave 2acegi.

Cyclohexylcarbamic acid 4-nitrophenyl-3-yl ester (2a): off-white solid; yield: 37% (0.255 g); mp: 134–136 °C (EtOH); MS (EI): m/z 215 (100); 1H NMR (CDCl3): δ = 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): v = 3321, 1716 cm⁻¹; Anal. calcd for C20H22N2O3 (338.41): C70.99, H6.55, N8.28, found: C71.05, H6.35, N8.23.

Cyclohexylcarbamic acid 6-nitrobiphenyl-3-yl ester (2c): white needles; yield: 85% (0.581 g); mp: 132–134 °C (EtOH); MS (EI): m/z 215 [M⁻], 198 (100); 1H NMR (CDCl3): δ = 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): v = 3322, 1717 cm⁻¹; Anal. calcd for C21H26N2O2 (340.48): C71.05, H6.59, N8.23.

Cyclohexylcarbamic acid 6-methylbiphenyl-3-yl ester (2e): white needles; yield: 6% (0.041 g); mp: 209–211 °C (EtOAc); MS (EI): m/z 338 [M⁺], 197 (100); 1H NMR (CDCl3): δ = 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): v = 3322, 1705 cm⁻¹; Anal. calcd for C21H25NO3 (338.41): C70.99, H6.65, N8.28, found: C71.07, H6.59, N8.33.

Cyclohexylcarbamic acid 6-carbamoylbiphenyl-3-yl ester (2f): white scales; yield: 90% (0.554 g); mp: 153–155 °C (EtOH); MS (EI): m/z 310 [M⁺], 184 (100); 1H NMR (CDCl3): δ = 1.12–2.06 (m, 10H), 2.25 (s, 3H), 3.55 (m, 1H), 4.90 (br d, 1H), 7.01–7.36 ppm (m, 8H); IR (Nujol): v = 3321, 1702 cm⁻¹; Anal. calcd for C21H25NO3·0.1CH2Cl2 (339.41): C72.71, H7.49, N4.53, found: C72.71, H7.40, N4.66.

Cyclohexylcarbamic acid 6-hydroxybiphenyl-3-yl ester (2g): white crystals; yield: 35% (0.226 g); mp: 118–119 °C (CHCl3/petroleum ether); MS (EI): m/z 251 [M⁺], 186 (100); 1H NMR (CDCl3): δ = 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): v = 3342, 1704 cm⁻¹; Anal. calcd for C21H17NO2·0.1CH2Cl2 (319.87): C71.72, H6.68, N4.38, found: C71.45, H6.59, N4.56.

Cyclohexylcarbamic acid 6-dimethylaminobiphenyl-3-yl ester (2h): white needles; yield: 98% (0.661 g); mp: 153–154 °C (cyclohexane); MS (EI): m/z 338 [M⁺], 213 (100); 1H NMR (CDCl3): δ = 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): v = 3310, 1710 cm⁻¹; Anal. calcd for C29H24N2O2 (338.45): C74.53, H7.74, N8.28, found: C74.97, H7.81, N8.40.
Cyclohexylcarboxylic acid 6-(3-cyclohexylureido)carbonyl)biphenyl-3-yl ester (2j): isolated as a side product of 2e; white crystals; yield: 45% (0.016 g); mp: 165–169°C (EtOAc); MS (EI): m/z 310 (M+1); 1H NMR (CDCl3): 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): ν = 3307, 1697 cm⁻¹. Anal. calcd for C₃₀H₂₅NO₂·0.5H₂O (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 CH₂Cl₂ (30 mL) under N₂ atmosphere was added to a stirred, cooled (0°C), solution of BB₃ (1 m, 6.5 mL) in CH₂Cl₂. The mixture was stirred at room temperature for the appropriate time (1 h for 1a; 20 h for 1e) then quenched with Na₂CO₃ (2 n) and extracted with EtOAc. The combined organic phases were dried (Na₂SO₄) and concentrated. Purification of the residue by column chromatography (cyclohexane/CH₂Cl₂ 7:3 for 1a, EtOAc for 1e) gave 1a–e as solids.

4-Nitrobiphenyl-3-ol (1a):[32] yellow crystals; yield: 83% (0.445 g); mp: 101–103°C (EtOH) [lit.: 104–105°C (EtOH)];[33] MS (EI): m/z 215 (M+1), 185, 168, 157, 139 (100), 128, 115, 77, 69, 63; 1H NMR (CDCl₃): δ = 7.21–7.66 (m, 7H), 8.18 (d, 1H), 10.72 ppm (s, 1H); IR (Nujol): ν = 3207 cm⁻¹.

5-Hydroxybiphenyl-2-carboxylic acid amide (1i): pale brown solid; yield: 98% (0.521 g); mp: 143–145°C (EtOAc); MS (EI): m/z 213 (M+1), 98, 186, 170, 139, 131, 128, 115, 84, 77, 69, 63, 56 (100); 1H NMR (CDCl₃): δ = 5.20 (br s, 1H), 5.46 (br s, 1H), 6.85 (m, 2H), 7.36 (m, 2H), 7.94 ppm (d, 1H).

Synthesis of 6-nitrobiphenyl-3-ol (1c):[31] LCI (1.9 g, 45 mmol) was added to a stirred solution of 8c (3.437 g, 15 mmol) in DMF (35 mL). After keeping the reagents at reflux for 24 h, a further amount of LCI (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 Et₂O, acidified with HCl (37%), and extracted again with Et₂O. The combined organic layers were dried (Na₂SO₄) 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+1), 198, 186, 170, 139, 131, 128, 115, 84, 77, 69, 63, 56 (100); 1H NMR (CDCl₃): δ = 5.80 (s, 1H), 6.86 (m, 2H), 7.36 (m, 2H), 7.94 ppm (d, 1H).

Synthesis of 3'-phenoxybiphenyl-3-ol (1l):[31] HCl (0.691 g, 2.5 mmol) and HCl (57%, 3.2 mL) were held at reflux under vigorous stirring for 3 h. The mixture was cooled, diluted with H₂O, neutralized with NaHCO₃ (2 n), and extracted with CH₂Cl₂. The combined organic phases were washed with a saturated aqueous solution of NaHCO₃ and brine, dried (Na₂SO₄), and concentrated. Purification of the residue by column chromatography (cyclohexane/CH₂Cl₂ 5:1) gave 1l as a pale yellow oil. Yield: 92% (0.660 g); MS (EI): m/z 262 (M+1), 115 (100); 1H NMR (CDCl₃): δ = 5.24 (s, 1H), 6.62 (m, 3H), 7.10–7.26 ppm (m, 10H); IR (neat): ν = 3391 cm⁻¹.

Synthesis of 6-aminobiphenyl-3-ol (1d):[34] HCl (37%, 27.5 mL) was added to a stirred suspension of 6c (2.367 g, 11 mmol) and Fe powder (1.535 g, 27.5 mmol) in dry CH₂Cl₂ (20 mL). The mixture was kept at reflux for 1.5 h, quenched with a solution of cooled saturated NaHCO₃, extracted with EtOAc, and washed with H₂O. The combined organic layers were dried (Na₂SO₄) and concentrated. Purification of the residue by column chromatography (cyclohexane/CH₂Cl₂ 6:4) and recrystallization from Et₂O gave 1d as an oil. Yield 25% (0.510 g); MS (EI): m/z 185 (M+1), 69, 57, 45, 31, 29, 27, 25, 23, 21, 19, 17, 15, 13, 11, 9, 7; IR (neat): ν = 3430 cm⁻¹.

Synthesis of 6-dimethylaminobiphenyl-3-ol (1d):[34] HCl (27.5%, 27.5 mL) was added to a stirred suspension of 1d (0.463 g, 2.5 mmol) in HCOOH (40%, 8.75 mL), and the mixture was kept at reflux overnight, cooled, neutralized with a saturated solution of NaHCO₃, and extracted with EtOAc. The combined organic layers were dried (Na₂SO₄) and evaporated. Purification of the residue by column chromatography (cyclohexane/CH₂Cl₂ 8:2) and recrystallization...
gave 1f as an off-white solid. Yield: 98% (0.520 g); mp: 70–72 °C (EtO/petroleum ether); MS (EI): m/z 213 (100); 1H NMR (CDCl3): δ = 2.55 (s, 6H), 6.77–7.57 ppm (m, 9H); IR (KBr): ν = 3425, 3039 cm⁻¹.

Synthesis of 1-phenyl-1H-indol-6-ol (1k): A solution of BBr₃ (1.4, 27.5 mL) in CH₂Cl₂ (3.32 g) was added to a stirred solution of 4 (5.852 g, 25 mmol) in dry CH₂Cl₂ (330 mL) under Ar atmosphere. The mixture was stirred at room temperature for 1 h, quenched with Na₂CO₃ (2.4 g), and extracted with EtOAc. The combined organic layers were washed with brine, dried (Na₂SO₄), 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-phenoxypentyl; MS (EI): m/z 276 (M+)]: 100; 1H NMR (CDCl3): δ = 3.80 (s, 3H), 6.36 (dd, 1H), 6.95 (dd, 1H), 7.40 (s, 5H), 7.44 (s, 5H), 8.37 ppm (d, 1H); IR (KBr): ν = 3417, 1644 cm⁻¹.

6-Methylbiphenyl-3-ol (1f):[26] Amber oil; yield: 90% (0.827 g); MS (EI): m/z 184 (100); 1H NMR and IR spectra according to published data.[26]

3-Hydroxy-3-phenoxypentyl (8i):[17] white crystals; yield: 62% (0.859 g); mp: 55–57 °C (cyclohexane) [lit.: 41–42 °C];[27] MS (EI): m/z 276 (M+]: 77 (100); 1H NMR (CDCl3): δ = 3.80 (s, 3H), 6.63–6.70 (m, 3H), 7.01 (m, 1H), 7.20–7.27 ppm (m, 9H, 1H).

Synthesis of 2-(or 3)-methoxy-(2- or 4)-nitrobenzophenones (8a–c): A mixture of HNO₃ (65%, 7 mL) and H₂SO₄ (96%, 7 mL) were added to a stirred solution of 3-methoxy-2-nitrobenzophenone (1.122 g, 70 mmol) in CH₂Cl₂ (70 mL) at 0 °C over the course of 45 min. The mixture was stirred for 3 h, cooled with ice water, neutralized with NaHCO₃, and extracted with CH₂Cl₂. The combined organic layers were dried (Na₂SO₄) and concentrated. Purification of the residue by column chromatography (cyclohexane/EtOAc 200:1 → 100:1) for 1f, 9:1 for EtOAc for 8e) gave 8e as solids and 1f as an oil.

5-Methoxybiphenyl-2-carboxylic acid amide (8e):[26] white crystals; yield: 95% (1.081 g); mp: 178–180 °C (EtOH) [lit.: 182–183 °C];[28] MS (E): m/z 227 (M+]: 139 (100); 1H NMR (CDCl3): δ = 3.87 (s, 3H), 5.14 (brs, 1H), 5.36 (brs, 1H), 6.83 (d, 1H), 6.95 (dd, 1H), 7.44 (s, 5H), 7.85 ppm (d, 1H); IR (KBr): ν = 3417, 1644 cm⁻¹.

5-Methoxybiphenyl-2-carboxylic acid amide (8e):[26] white crystals; yield: 95% (1.081 g); mp: 178–180 °C (EtOH) [lit.: 182–183 °C];[28] MS (E): m/z 227 (M+]: 139 (100); 1H NMR (CDCl3): δ = 3.87 (s, 3H), 5.14 (brs, 1H), 5.36 (brs, 1H), 6.83 (d, 1H), 6.95 (dd, 1H), 7.44 (s, 5H), 7.85 ppm (d, 1H); IR (KBr): ν = 3417, 1644 cm⁻¹.

Synthesis of 6-methoxy-1-phenyl-1H-indole (4):[21] A mixture of 6-methoxy-1H-indole (3) (5.887 g, 40 mmol), CH₃I (14.117 g, 92.2 mmol), K₂CO₃ (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 Et₂O, and a dilute solution of NH₄OH. The organic layer was washed with brine, dried (Na₂SO₄), 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 (E): m/z 223 (M+]: 77 (100); 1H NMR (CDCl3): δ = 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): SOCl₂ (1.428 g, 0.875 mL, 12 mmol) was added to a stirred suspension of 2-bromo-4-methoxybenzonic 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 CH₂Cl₂ (20 mL) 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 (E): m/z 230 (M+]: 213 (100); 1H NMR (CDCl3): δ = 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): ν = 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 were taken from the Hansch collection.[14] 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 q² 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).[15]

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 force field implemented in Sybyl 6.9.[40] The resulting structures were used as starting input structures for DFT[40] calculations with the B3LYP[40–42] 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: 1MTS),[29] a functional subunit of the FAAH enzyme was prepared as described[29] 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 oxygen 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[22] 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 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 [3H]anandamide (anandamide[ethanolamine–H]), 60 Cimmol[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), [3H]anandamide (10000 dpm, 10 nM), and were stopped with a mixture of chloroform/methanol and acid-free bovine serum albumin (0.05% by weight), and were conducted for a further 30 min.

[3H]anandamide (10000 dpm, 10 nM) was added, and the reactions were stopped with a mixture of chloroform/methanol and acid-free bovine serum albumin (0.05% by weight), and were conducted for a further 30 min.

Acknowledgments

This work was supported by MIUR (Ministero dell’Istruzione, dell’Università e della Ricerca), the Universities of Parma and Urbino “Carlo Bo”, and the National Institute on Drug Abuse (NIDA). The CCE (Centro di Calcolo Elettronico) and CIM (Centro Interfacoltà Misure) of the University of Parma are gratefully acknowledged for supplying the Sybyl software license.

Keywords: carbamate inhibitors · FAAH · molecular modeling · structure–activity relationships · UR8524

[1] D. Piomelli, Nat. Rev. Neurosci. 2003, 4, 873–884.
[2] a) A. G. Hohmann , R. L. Supilia II, N. N. Bolton, M. H. Neely, D. Fegley, R. Mangieri, J. Krey, J. M. Walker, P. V. Pizzi, B. F. Cravatt, J. Med. Chem. 2003, 46, 2352–2360.
[3] b) J. K. Makara, M. Mor, D. Fegley, S. I. Szabó, S. Kathuria, G. Astarita, A. Duranti, A. Tontini, M. Mor, G. Tarzia, G. La Rana, A. Calignano, A. Giustino, M. Tattoli, M. Palmery, V. Cuomo, D. Piomelli, Nat. Med. 2003, 9, 76–81.
[4] a) D. L. Boger, H. Miyauchi, W. Du, C. Hardouin, R. A. Fecik, H. Cheng, I. Hwang, M. P. Hedrick, D. Leung, O. Acevedo, C. R. W. Guimaraes, W. L. Jorgensen, B. F. Cravatt, J. Med. Chem. 2005, 48, 1849–1856.
[5] a) D. L. Boger, H. Miyauchi, W. Du, C. Hardouin, H. Cheng, I. Hwang, B. F. Cravatt, D. L. Boger, Bioorg. Med. Chem. Lett. 2005, 15, 1423–1428.
[6] a) D. L. Boger, H. Miyauchi, W. Du, C. Hardouin, R. A. Fecik, H. Cheng, I. Hwang, M. P. Hedrick, D. Leung, O. Acevedo, C. R. W. Guimaraes, W. L. Jorgensen, B. F. Cravatt, J. Med. Chem. 2005, 46, 2352–2360.
[7] a) B. Koutek, G. D. Prestwich, A. C. Howlett, S. A. Chin, D. Salehani, N. Akhavan, D. G. Deutsch, J. Biol. Chem. 1994, 269, 22937–22940; b) E. J. Patterson, I. R. Ollmann, B. F. Cravatt, D. L. Boger, C.-H. Wong, R. A. Lerner, J. Am. Chem. Soc. 1996, 118, 5938–5945; c) D. L. Boger, H. Sato, A. E. Lerner, B. J. Austin, J. E. Patterson, M. P. Patricelli, B. F. Cravatt, Bioorg. Med. Chem. Lett. 1999, 9, 265–270; d) D. L. Boger, H. Sato, A. E. Lerner, M. P. Hedrick, R. A. Fecik, H. Miyauchi, G. D. Willie, B. J. Austin, M. P. Patricelli, B. F. Cravatt, Proc. Natl. Acad. Sci. USA 2000, 97, 5044–5049; e) D. L. Boger, H. Miyauchi, M. P. Hedrick, Bioorg. Med. Chem. Lett. 2001, 11, 1517–1520.
[8] a) A. Abouabedallah, M. Bas, G. Dargazani, C. Hoornaert, A. T. Li, F. Medaisko, WO Patent 020430, 2004; b) A. Abouabedallah, P. Burnier, C. Hoornaert, J. Jeunesse, F. Puech, WO Patent 099176, 2004; c) S-Y. Sit, K. Xie, WO Patent 087569, 2002; d) S-Y. Sit, K. Xie, H. Deng, WO Patent 055989, 2003.
[9] a) J. O. Pritchard, C. L. Crawford, S. R. Crowley, S. Maurelli, Y. Kurahashi, S. Yamamoto, G. Marino, V. Di Marzo, Biochem. Biophys. Res. Commun. 1997, 231, 82–88.
[10] a) D. L. Boger, H. Miyauchi, W. Du, C. Hardouin, R. A. Fecik, H. Cheng, I. Hwang, M. P. Hedrick, D. Leung, O. Acevedo, C. R. W. Guimaraes, W. L. Jorgensen, B. F. Cravatt, J. Med. Chem. 2005, 46, 2352–2360.
[11] For some experiments, brain membranes were pre-incubated at 37 °C for various durations with test compounds. Reactions were started by adding [3H]anandamide and were conducted for a further 30 min.

In conclusion, the principle leading to 29 may also have produced the regioisomeric ortho-carbamoylated biphenyl 9. Unequivocal structure determination was carried out with two independent NMR experiments:
nuclear Overhauser enhancement (nOe) and heteronuclear multiple-bond correlation (HMBC). Concerning nOe, selective irradiation of OH is expected to produce an enhancement of signals for H3, H2, and H6 in isomer 2g, and for H1 and H5 in the case of 9. In fact, we observed enhancements of 4.1% for H2 and H6 and of 3.3% for H3, whereas enhancements of the signals for H1 and H5 were negligible (data not shown). Concerning HMBC, heteronuclear long-range couplings of OH are expected with Cipso and the two Cortho atoms, corresponding to C2, C1, and C3 in isomer 2g, and to C5, C4, and C6 in isomer 9. The observed long-range correlations again confirmed structure 2g (data not shown).

[33] J. C. Colbert, D. W. Fox, C. Matuszak, J. Am. Chem. Soc. 1955, 77, 2447–2450.
[34] A. Avenoza, J. H. Busto, C. Cativiela, J. M. Peregrina, Synthesis 1995, 671–674.
[35] G. Tsatsas, A. Psarrea-Sandris, C. Sandris, J. Med. Chem. 1967, 10, 489–491.
[36] T. Matsusura, J.-B. Meng, Y. Ito, M. Irie, K. Fukuyama, Tetrahedron 1987, 43, 2451–2456.
[37] T. Goodson Jr., R. W. Harper, D. K. Herron, WO Patent 24794, 1998.
[38] J. E. Simpson, G. H. Daub, F. N. Hayes, J. Org. Chem. 1973, 38, 4428.
[39] H.-J. Knölkert, E. Baum, T. Hopffmann, Tetrahedron 1999, 55, 10391–10412.
[40] P. A. S. Smith, J. H. Hall, J. Am. Chem. Soc. 1962, 84, 480–485.
[41] M. R. Marzabadi, J. Wetzel, J. E. Deleon, Y. Jiang, C.-A. Chen, K. Lu, US Patent 6727264, 2004.
[42] C. Hansch, A. Leo, D. Hoekman, Exploring QSAR: Hydrophobic, Electronic and Steric Constants; American Chemical Society, Washington, DC, 1995.
[43] S. Wold, L. Eriksson in Chemometric Methods in Molecular Design (Ed.: H. van de Waterbeemd), VCH Publishers, New York, 1995, pp. 309–318.
[44] T. A. Halgren, J. Comput. Chem. 1999, 20, 720–729.
[45] Sibyl 6.9, Tripos Inc., 1699 South Hanley Rd., St. Louis, Missouri, 63144, USA.
[46] R. G. Par, W. Yang, Density-functional theory of atoms and molecules; Oxford University Press, Oxford, 1989.
[47] A. D. Becke, J. Chem. Phys. 1993, 98, 5648–5652.
[48] C. Lee, W. Yang, R. G. Parr, Phys. Rev. B 1988, 37, 785–789.
[49] S. H. Vosko, L. Wilk, M. Nusair, Can. J. Phys. 1980, 58, 1200–1211.
[50] P. J. Stephens, F. J. Devlin, C. F. Chabalowski, M. J. Frisch, J. Phys. Chem. 1994, 98, 11623–11627.
[51] Jaguar 4.2, Schrödinger, Inc., Portland, OR, 1991.
[52] F. Mohamadi, N. G. J. Richards, W. C. Guida, R. Liskamp, M. Lipton, C. Caufield, G. Chang, T. Hendrickson, W. C. Still, J. Comput. Chem. 1990, 11, 440–467.