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O-(Triazolyl)methyl Carbamates as a Novel and Potent Class of Fatty Acid Amide Hydrolase (FAAH) Inhibitors

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Inhibition of fatty acid amide hydrolase (FAAH) activity is under investigation as a valuable strategy for the treatment of several disorders, including pain and drug addiction. A number of potent FAAH inhibitors belonging to different chemical classes have been disclosed to date; *O*-aryl carbamates are one of the most representative families. In the search for novel FAAH inhibitors, a series of *O*-(1,2,3-triazol-4-yl)methyl carbamate derivatives were designed and synthesized exploiting a copper-catalyzed [3+2] cycloaddition reaction between azides and alkynes (click chemistry). Exploration of the structure–activity relationships within this new class of compounds identified potent inhibitors of both rat and human FAAH with IC_{50} values in the single-digit nanomolar range. In addition, these derivatives showed improved stability in rat plasma and kinetic solubility in buffer with respect to the lead compound. Based on the results of the study, the novel analogues identified can be considered to be promising starting point for the development of new FAAH inhibitors with improved drug-like properties.

Introduction

Fatty acid amide hydrolase (FAAH)[1] is a membrane-bound serine hydrolase that catalyzes the hydrolytic cleavage of endogenous biologically active fatty acid ethanolamides (FAEs), such as anandamide (AEA), an agonist of cannabinoid receptors,[2] and palmitoylethanolamide (PEA),[3] and oleoylethanolamide (OEA),[4] which are agonists of type-α peroxisome proliferator-activated receptors (PPAR-α).[5] These natural FAAH substrates may play important roles in the central nervous system (CNS) and in peripheral tissues, where they are involved in a variety of physiological processes.[6]

Substantial efforts have been dedicated to the discovery of potent and selective FAAH inhibitors, with the objective of developing therapeutic approaches for pathologic conditions such as pain, drug addiction, anxiety, and depression.[6,7] Different classes of molecules are known to increase intracellular FAE levels through FAAH inhibition, including carbamates[8] and piperidine/piperazine ureas[9] that covalently bind to FAAH,[10] and α-keto heterocycle-based inhibitors,[11] which inhibit FAAH by reversible hemiketal formation with the catalytic serine of the enzyme.[12] Among them, the *O*-arylcarbamate series (Figure 1), exemplified by URB524 (**1a**),[8a,b,13] URB597 (**1b**),[8a,b,13] and URB694 (**1c**),[8c,14] has been extensively investigated.[15] In particular, it was shown that compound **1b** exerts a combination of anxiolytic-like, anti-depressant-like, and analgesic effects, because of its ability to inhibit FAAH activity in the CNS and peripheral tissues.[16]

The active site of FAAH is characterized by an atypical catalytic triad, consisting of Ser241–Ser217–Lys142, which is capable of hydrolyzing amide and ester bonds at similar rates.[17] Several studies, including computational modeling,[18] supported by the resolution of the crystal structure of humanized rat FAAH in complex with **1b**,[19] indicate that *O*-arylcarbamates bind covalently to FAAH and cause its irreversible inhibition. In particular, it has been proposed that this class of molecules is attacked at the carbonyl group by Ser241, leading to the formation of carbamoylated, catalytically inactive FAAH and releasing the *O*-biphenyl moiety as the leaving group.

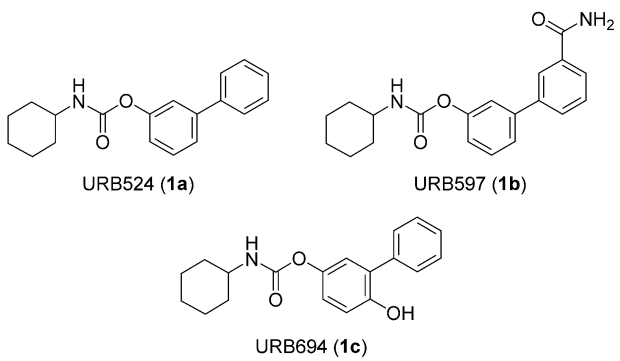

Figure 1. *O*-Arylcarbamates fatty acid amide hydrolase (FAAH) inhibitors.

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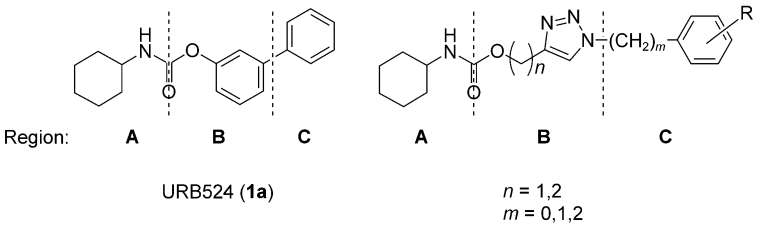


Figure 2. Side-by-side comparison of URB524 (**1a**) and *O*-(1,2,3-triazol-4-yl)alkyl carbamate derivatives.

O-Arylcarbamates such as **1b** are selective for FAAH but can also interact with select liver carboxylesterases, at least at high concentrations, and have limited plasma stability.[9a] Recently, however, highly potent *O*-arylcarbamates with markedly improved selectivity for FAAH were identified.[8c] The insertion of an electron-donating substituent, such as a hydroxy or amino group, in the *para* position of the proximal phenyl ring of **1a** did not significantly affect inhibitory potency in vitro, but caused a marked increase in the stability of the compounds in plasma, in comparison to other molecules in the series. URB694 (**1c**) was identified as a potent FAAH inhibitor with improved plasma stability, prolonged half-life in vivo, and decreased activity towards liver carboxylesterases in comparison to **1b**.

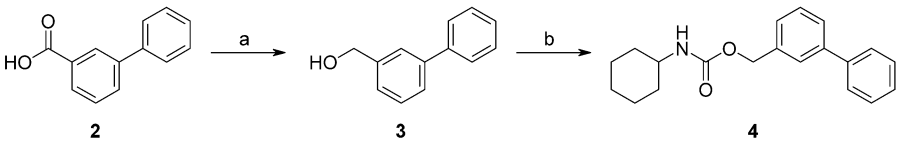
In the search for better FAAH inhibitors with improved stability, we designed a novel class of carbamates where the *O*-aryl moiety is replaced by an *O*-(triazol-4-yl)alkyl group (Figure 2). We expected those compounds to be more stable than their *O*-aryl analogues, since the aliphatic alcohol resulting from the nucleophilic attack on the carbamate is a poorer leaving group than a phenol. Although carbamate-based FAAH inhibitors containing an *O*-(heteroaryl)alkyl moiety have been reported in the patent literature,[12] to the best of our knowledge, no *O*-(triazol-4-yl)alkyl carbamate derivatives have been described to date.

Herein, we report the synthesis and characterization of a series of *O*-(1,2,3-triazol-4-yl)alkyl carbamates, prepared by copper-catalyzed [3+2] cycloaddition reaction between azides and alkynes.[20] The fast and versatile synthesis via click chemistry allowed us to prepare a number of analogues in a quick and reliable manner, and rapidly explore the SAR within this new class of FAAH inhibitors.

Results and Discussion

Chemistry

(3-Phenylphenyl)methyl *N*-cyclohexylcarbamate (**4**) was prepared from commercially available 3-phenylbenzoic acid (**2**) by lithium aluminum hydride reduction to 3-phenylbenzyl alcohol (**3**) followed by reaction with commercial cyclohexyl isocyanate (Scheme 1).



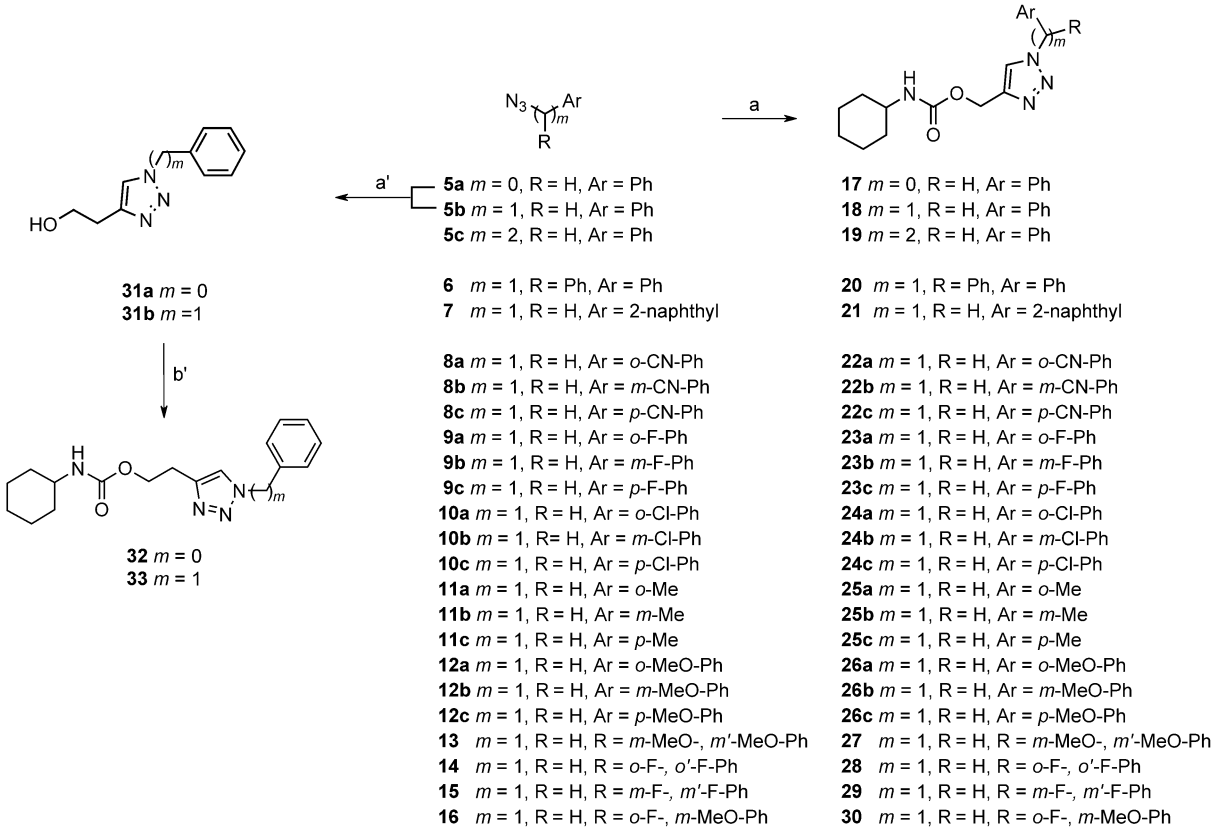
Scheme 1. Synthesis of (3-phenylphenyl)methyl *N*-cyclohexylcarbamate (**4**). *Reagents and conditions*: a) $LiAlH_4$ (2 N in THF), dry THF, 0 °C→rt, 2 h; b) cyclohexyl isocyanate, DMAP, dry CH_3CN, 80 °C, 6 h.

The preparation of 1,4-disubstituted-1,2,3-triazoles was accomplished by [3+2] cycloaddition reaction between azides and alkynes, in the presence of copper(I) salts (click chemistry).[20] We designed a versatile synthetic strategy that allowed us to generate a first set of molecules, bearing an *O*-(1,2,3-triazol-4-yl)methyl moiety. The desired compounds (**17**–**30**, **32**, **33**) were synthesized as shown in Scheme 2.

Aromatic azide **5a** was prepared from aniline by a diazotation–azidation protocol,[21] while **5b**, **5c**, and **6**–**16** were obtained in good to excellent yields by reacting the corresponding halides with sodium azide.[22] Final compounds **17**, **18**, and **19** were prepared via click chemistry, starting from prop-2-yn-1-yl *N*-cyclohexylcarbamate,[23] prepared by reaction of cyclohexylamine with the commercially available prop-2-ynyl chloroformate, and azides **5a**, **5b**, and **5c**, respectively (Scheme 2). Then, copper catalyzed [3+2] cycloaddition reaction between azides **5a** and **5b** with the commercially available but-3-yn-1-ol, allowed us to obtain compounds **31a** and **31b** in acceptable yields.[24] Finally, compounds **32** and **33** were prepared by coupling alcohols **31a** and **31b**, respectively, with commercial cyclohexyl isocyanate (Scheme 2). *O*-(1,2,3-Triazol-4-yl)methyl carbamate derivatives **20**–**30** (Scheme 2) were prepared by reaction of azides **6**–**16** with prop-2-yn-1-yl *N*-cyclohexylcarbamate, under click chemistry conditions.

A second set of analogues was synthesized, as reported in Scheme 3, in order to explore region A (Figure 2). Prop-2-ynyl-*N*-prop-2-ynyl carbamate,[25] obtained by reaction of the commercially available propargyl amine with prop-2-ynyl chloroformate, was reacted with aromatic azides **12a** and **12b** thus affording compounds **34a** and **34b**, which bear the same substituent on both aromatic rings (Scheme 3).

Bis-(triazol-4-yl)methyl carbamates with different substitution pattern on the aromatic rings were synthesized according to Scheme 3. Aromatic azides **12a**–**c** were reacted with propargyl amine, under click chemistry conditions, to give the corresponding aminomethyl triazoles (**35a**–**c**), which were subsequently coupled with prop-2-ynyl chloroformate to afford *N*-(triazol-4-yl)methyl-*O*-propargyl carbamates **36a**–**c**. The latter compounds were reacted with aromatic azides **12a**–**b**, under click chemistry conditions, to afford *N*-(triazol-4-yl)methyl-*O*-(triazol-4-yl)methyl carbamates **37**–**40**.



Scheme 2. Synthesis of *O*-(triazol-4-yl)alkyl carbamates **17**–**30**, **32** and **33**. *Reagents and conditions*: a) prop-2-yn-1-yl *N*-cyclohexylcarbamate, sodium L-ascorbate, $CuSO_4{\cdot}5H_2O$, H_2O/*t*-BuOH (1:1), rt, 3 h; a′) but-3-yn-1-ol, sodium L-ascorbate, $CuSO_4{\cdot}5H_2O$, H_2O/*t*-BuOH (1:1), rt, 3 h; b′) cyclohexyl isocyanate, DMAP, dry CH_3CN, 80 °C, 5 h.

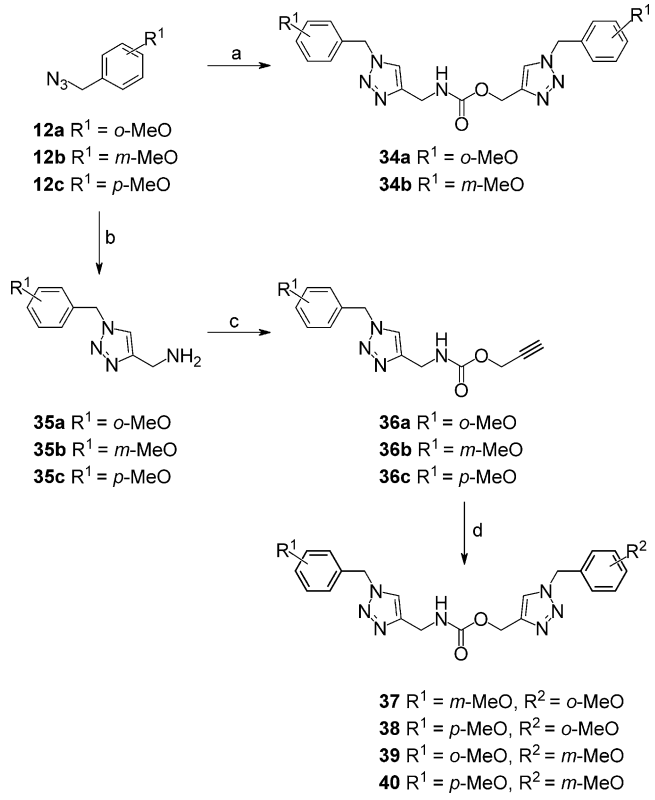
The compounds bearing a (1,2,4-triazol-3-yl-)methyl moiety, **43** and **46**, were synthesized as reported in Scheme 4. Compound **43** was prepared by lithium aluminum hydride reduction of commercially available 1-phenyl-1,2,4-triazole-3-carboxylic acid **41** followed by reaction with cyclohexyl isocyanate. Then, commercially available methyl *1H*-1,2,4-triazole-3-carboxylate **44** was reacted with benzyl bromide in the presence of potassium carbonate to obtain compound **45**. Reduction by lithium aluminum hydride followed by reaction with cyclohexyl isocyanate afforded compound **46**.

Structure–activity relationship (SAR) and stability studies

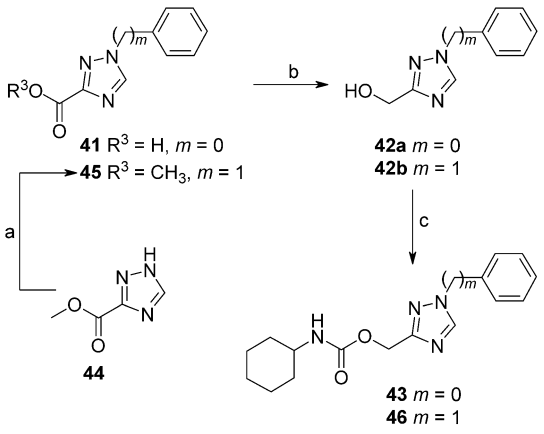
The compounds were tested for their ability to inhibit the hydrolysis of [^{3}H]anandamide by FAAH prepared from rat brain homogenates. Median inhibitory concentration (IC_{50}) values are reported in Tables 1–4. In a first attempt to improve the stability of *O*-biphenyl carbamate FAAH inhibitors, we replaced the *O*-(3-phenylphenyl) residue of **1a** with an *O*-(3-phenylphenyl)-methyl group, as in compound **4**. This change caused an almost complete loss of activity, as **4** showed only 65% inhibition of FAAH activity at 100 μM (Table 1). Interestingly, the substitution of the *O*-(3-phenylphenyl)methyl group with an *O*-(1-phenyl-1,2,3-triazol-4-yl)methyl residue, as in compound **17** (IC_{50} = 381 nM), recovered the FAAH inhibitory activity. Encouraged by this result, we synthesized a set of close analogues of **17**, compounds **18**–**19** and **32**–**33**, to identify the best substituents at positions 1 and 4 of the triazole ring for FAAH inhibition. The results are reported in Table 1.

Replacement of the phenyl group at position 1 of the triazole with a benzyl residue, compound **18** (IC_{50} = 26 nM), led to an approximate 15-fold increase in potency. It is interesting to note that this compound was only 4.6-fold less potent than URB524 (**1a**). When the methylene linker at position 1 or 4 of the triazole ring was substituted by an ethylene moiety, as in compounds **19** and **33**, respectively, a drop in potency with respect to **18** was observed. The replacement of the 4-methylene residue in compound **17** with a 4-ethylene one, leading to compound **32** (IC_{50} = 1278 nM), resulted in around a threefold decrease in potency.

We then investigated whether 1-phenyl- or 1-benzyl-(1,2,4-triazol-3yl)methyl moieties work as replacements of the *O*-(3-phenylphenyl) residue of **1a** and whether they are interchangeable with the isomeric 1-phenyl- and 1-benzyl-(1,2,3-triazol-4-yl)methyl residues. Compounds **43** and **46** showed a dramatic decrease in potency with respect to **1a**, and surprisingly, these compounds were much less potent than **17** and **18**. We speculate that the loss in potency might be ascribed to the different electronic properties of the 1,2,4-triazole ring with respect to the isomeric 1,2,3-triazole counterpart, leading to an unfavorable interaction with the active site of the enzyme.



Scheme 3. Syntheses of compounds **34a**,**b** and **37**–**40**. *Reagents and conditions*: a) **12a** or **12b**, prop-2-yn-1-yl *N*-(prop-2-yn-1-yl)carbamate, sodium L-ascorbate, $CuSO_4{\cdot}5H_2O$, H_2O/*t*-BuOH (1:1), rt, 3 h; b) prop-2-yn-1-amine, sodium L-ascorbate, $CuSO_4{\cdot}5H_2O$, H_2O/*t*-BuOH (1:1), rt, 3 h; c) prop-2-ynyl chloroformate, Et_3N, dry CH_2Cl_2, 0 °C, 30 min; d) **12a** or **12b**, sodium L-ascorbate, $CuSO_4{\cdot}5H_2O$, H_2O/*t*-BuOH (1:1), rt, 3 h.



Scheme 4. Syntheses of (1,2,4-triazol-3-yl)methyl *N*-cyclohexylcarbamates **43**and **46**. *Reagents and conditions*: a) BnBr, K_2CO_3, dry DMF, 80 °C, 16 h; b) $LiAlH_4$ (2 N in THF), dry THF, 0 °C→rt, 2 h; c) cyclohexyl isocyanate, DMAP, dry CH_3CN, 80 °C, 6 h.

To test our hypothesis that *O*-(triazol-4-yl)alkyl carbamate derivatives are more stable than *O*-aryl carbamates, we compared the rat plasma stability of compounds **1a**, **17** and **18**. The results are reported in Table 1. Consistent with our expectation, *O*-(1,2,3-triazol-4-yl)methyl carbamates **17** and **18** showed significantly higher plasma stability than **1a**. The latter compound displayed a half-life of 62 min,[26] but was no longer detectable after 7 h incubation with rat plasma. In contrast, approximately 90% of the initial amount of compound **17** and **18** was still detectable after 7 h.

The limited decrease in potency of compound **18** versus **1a**, coupled with its significantly higher plasma stability, prompted us to explore further this chemical class. We first investigated region C (Figure 2) by preparing a series of compounds bearing variously substituted benzyl residues at position 1 of the triazole ring. The results are summarized in Table 2.

Replacement of the benzyl residue in **18** with a benzhydryl moiety, compound **20** (73% inhibition at 100 μM), or a 2-naphthylmethyl group, compound **21** (IC_{50} = 2.0 μM), led to a significant decrease in FAAH inhibitory potency, indicating that bulky arylmethyl groups linked to the triazole are not tolerated, most likely because of steric clash at the active site of the enzyme.

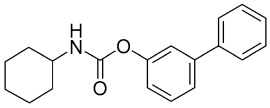
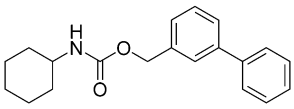
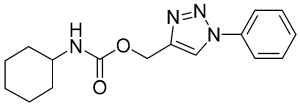
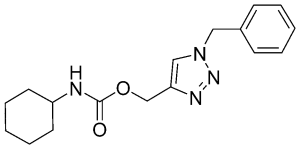
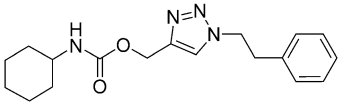
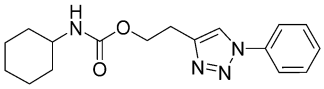
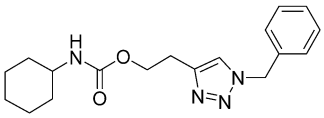
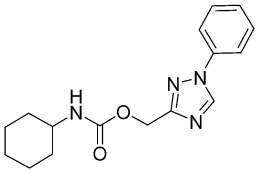
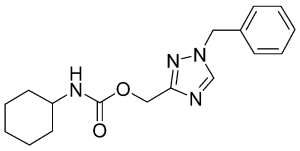
Interestingly, the nature of the substituent on the phenyl ring appeared to have a limited effect on the potency of the compounds as FAAH inhibitors. In fact, benzyl residues bearing both electron-withdrawing (CN, F, Cl) and electron-donating (Me, OMe) substituents at the *ortho* or *meta* position all led to low-nanomolar inhibitors. Among them, *ortho*-methoxybenzyl derivative **26a** showed the highest potency (IC_{50} = 1.4 nM). All of the *para*-substituted derivatives were less potent than the corresponding *ortho*- or *meta*-substituted analogues, irrespective of the electronic properties of the substituent. In particular, compound **22c** (IC_{50} = 2282 nM), bearing a *para*-cyano group, showed the highest loss in potency within this subset of analogues. As for the naphthylmethyl compound (**21**), we interpret this finding as the result of an unfavorable steric interaction between the *para*-substituted phenyl ring and the active site of the enzyme. Together, from this small series of derivatives, the rank order of potency *ortho*- > *meta*- > *para*-substituted compounds clearly emerged.

The excellent potency of benzyl derivatives bearing a fluoro or a methoxyl group at the *ortho* or *meta* position led us to synthesize di-substituted compounds **27**–**30** to verify whether any additive effect on potency was observed. With the exception of 2-fluoro-3-methoxy-derivative **30** (IC_{50} = 44.6 nM), all of the compounds retained an excellent potency, with IC_{50} values in the range 10.4–11.9 nM, but none of them improved significantly over the corresponding mono-substituted analogue.

The most potent compound, **26a**, was effective at inhibiting FAAH activity ex vivo. One hour after systemic administration of **26a** (3 mg kg^{-1}, intraperitoneally) to CD1 mice, FAAH activity measured ex vivo in brain tissue was reduced by 78% (n = 3) with respect to the control.

As the next step in the investigation of the SAR of this new class of FAAH inhibitors, we conducted a preliminary exploration of region A (Figure 2). Previous studies on *O*-arylcarbamates showed that replacement of the cyclohexyl group of **1a** with an arylalkyl moiety led to inhibitors of greater potency.[15a] Exploiting the click chemistry approach, we replaced the cyclohexyl group with a [1-(methoxybenzyl)triazol-4-yl]methyl residue, as in compounds **34a** and **34b**, and **37**–**40**. The results are reported in Table 3.

Table 1. Fatty acid amide hydrolase (FAAH) inhibitory activity and rat plasma stability of **1a**, **4**, and *O*-[1-substituted-(1,2,3-triazol-4-yl)]alkyl carbamates.

Compd	Structure	IC_{50} [nM][a]	Plasma $t_{1/2}$ [min]	Plasma stability [% at 7 h][b]
1a		5.7 ± 1.2	62 ± 19	0
4		65%@100 μM	–	–
17		380.6 ± 62.9	–	90 ± 9
18		26.2 ± 3.8	–	92 ± 8
19		833 ± 129	–	–
32		1278 ± 84	–	–
33		2535 ± 106	–	–
43		70%@100 μM	–	–
46		51 500 ± 1125	–	–

[a] IC_{50} values are the mean ± SEM ($n = 3$). [b] Percent of parent compound remaining after 7 h incubation.

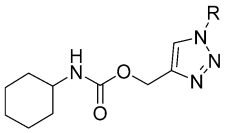
Compound **34a**, bearing an *ortho*-methoxybenzyl residue on both triazolyl rings turned out to be the least potent derivative (IC_{50} = 154 nM). Moving the methoxyl group to the *meta* position of the *N*-(1-benzyltriazol-4-yl)methyl moiety led to derivative **37** (IC_{50} = 3.2 nM), which showed a 48-fold increase in potency versus **34a** and confirmed as the most potent compound of this small series. Consistent with the previous finding, replacement of the *ortho*-methoxybenzyl residue at position 1 of the triazole ring in region C with a *meta*-methoxybenzyl group, as in compound **39** (IC_{50} = 9.8 nM), was accompanied by an approximate 15-fold increase in potency with respect to **34a**. Introduction of a *meta*-methoxybenzyl group at position 1 on both triazolyl rings led to the potent inhibitor **34b** (IC_{50} = 3.9 nM). Interestingly, a *para*-methoxybenzyl group at position 1 of the (triazol-4-yl)methyl moiety in region A was not detrimental for potency, as compounds **38** and **40** inhibited FAAH activity with IC_{50} values of 7.6 and 5.8 nM, respectively.

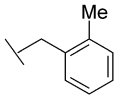
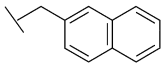
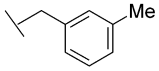
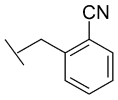
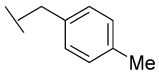
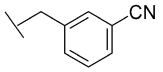
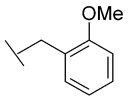
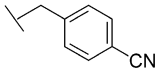
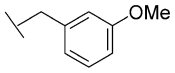
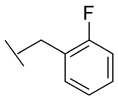
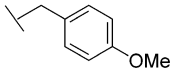
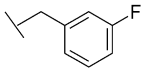
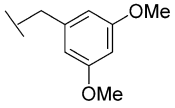
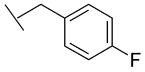
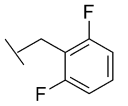
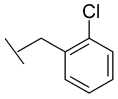
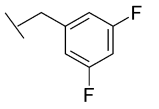
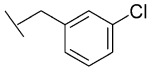
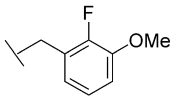
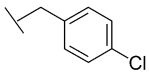
The most interesting compounds identified from the SAR exploration were tested for their inhibitory activity against human (*h*)-FAAH-1. A comparison between the inhibitory potency of selected compounds on rat (*r*)-FAAH versus *h*-FAAH-1 is reported in Table 4.

The series of substituted *O*-(1-benzyltriazol-4-yl)methyl *N*-cyclohexylcarbamate derivatives resulted to be generally less active at inhibiting *h*-FAAH-1 than *r*-FAAH, displaying a 14- to 240-fold drop in potency. The only exception was **27** (IC_{50} = 3.6 nM), which showed threefold higher potency on *h*-FAAH-1 with respect to *r*-FAAH. Moreover, *ortho*-substituted analogues **23a**, **24a**, and **26a**, which displayed single-digit nanomolar *r*-FAAH inhibition, and 2,6-dibustituted derivative **28**, suffered the most marked loss of potency: 62, 172, 240, and 73-fold respectively. *meta*-Substituted analogues **23b**, **24b**, and **26b**, and 3,5-disubstituted derivative **29** were more potent inhibitors than their *ortho*-substituted analogues, thus reversing the preference for *ortho*-substituted benzyl residues observed with *r*-FAAH.

By contrast, compounds bearing substituted (1-benzyltriazol-4-yl)methyl residues at both region A and C (Figure 2) displayed a minor decrease in activity on *h*-FAAH-1 compared to the *N*-cyclohexylcarbamate derivatives. Indeed, compounds **37**, **38**, and **39** showed four- to tenfold lower potency, but retained double-digit nanomolar potency on *h*-FAAH-1. The most promising molecules in terms of potency were **34b** and **40**, which possess IC_{50} values for *h*-FAAH-1 inhibition of 4.2 nM and 9.4 nM, respectively. Interestingly, both of them are charac-

Table 2. Fatty acid amide hydrolase (FAAH) inhibitory activity of *O*-(triazol-4-yl)methyl carbamates variously substituted at region C.



Compd	R	IC_{50} [nM][a]	Compd	R	IC_{50} [nM][a]
20		73%@100 μM	**25a**		21.5 ± 1.2
21		2003 ± 636	**25b**		24.5 ± 6.2
22a		19.5 ± 4.3	**25c**		500 ± 44
22b		21.5 ± 7.9	**26a**		1.4 ± 0.2
22c		2282 ± 585	**26b**		11.8 ± 3.7
23a		9.9 ± 1.9	**26c**		627 ± 173
23b		12.1 ± 0.8	**27**		10.5 ± 2.9
23c		95.5 ± 22.2	**28**		11.9 ± 1.0
24a		6.2 ± 1.3	**29**		10.4 ± 2.6
24b		13.2 ± 2.8	**30**		44.6 ± 10.3
24c		215 ± 31			

[a] IC_{50} values are the mean ± SEM ($n = 3$).

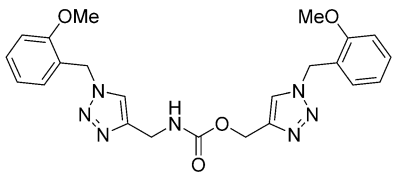
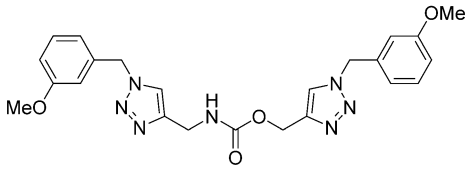
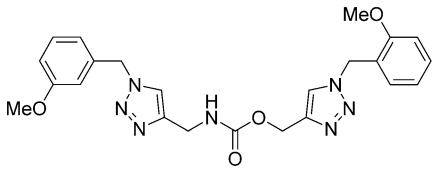
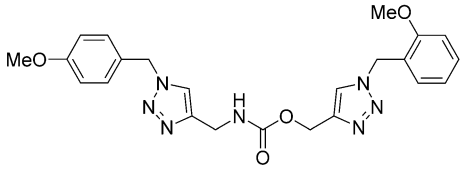
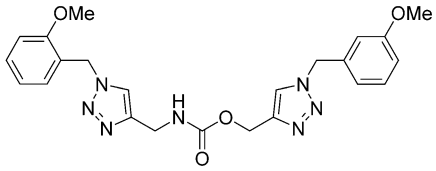
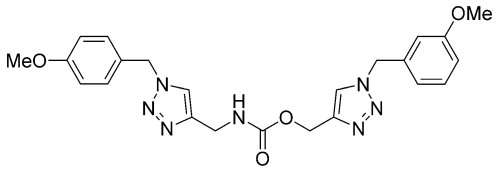
terized by a *meta*-methoxybenzyl group on region C (Figure 2), suggesting that the binding site on *h*-FAAH-1 prefers *meta*-substituents on that benzyl group.

The most potent compounds against both rat and human FAAH, that is, **27**, **34b**, and **40**, were tested for their selectivity versus monoacylglycerol lipase (MGL), a serine hydrolase that inactivates the endocannabinoid 2-arachidonoylglycerol (2-AG).[27] None of the compounds inhibited MGL activity when tested at concentrations up to 100 μM (Figure S1 in the Supporting Information). The selective inhibition of FAAH activity by compounds **27**, **34b**, and **40** is in agreement with previous observations with *O*-arylcarbamates **1a** and **1b**.[28]

Finally, compounds **27**, **34b**, and **40** were further characterized by determining their rat plasma and mouse liver microsomal (MLM) stability, and their kinetic solubility in buffer. A comparison of the overall profile of *O*-arylcarbamate **1a** with compounds **27**, **34b**, and **40** is reported in Table 5.

The selected *O*-[(1-benzyltriazol-4-yl)methyl]carbamate derivatives **27**, **34b**, and **40** showed FAAH inhibitory activity and MLM stability comparable to those of *O*-arylcarbamate **1a**.

Table 3. Fatty acid amide hydrolase (FAAH) inhibitory activity of *N*-(triazol-4-yl)methyl *O*-(triazol-4-yl)methyl carbamates **34a–40**

Compd	Structure	IC_{50} [nM][a]
34a		154 ± 14
34b		3.9 ± 0.7
37		3.2 ± 1.2
38		7.6 ± 0.6
39		9.8 ± 0.1
40		5.8 ± 0.6

[a] IC_{50} values are the mean ± SEM ($n = 3$).

However, they displayed much higher rat plasma stability than **1a**, as approximately 90% of the initial amount of the compounds was still present after 7 h incubation with rat plasma. Moreover, with the exception of compound **27**, the kinetic solubility in buffer also improved significantly.

Conclusions

In the present study, we report the synthesis and characterization of *O*-(1,2,3-triazol-4-yl)alkyl carbamates as a novel class of FAAH inhibitors. In these compounds, an *O*-(triazol-4-yl)methyl group replaces the *O*-aryl moiety of known and potent FAAH inhibitors such as compound **1a** (URB524) and **1b** (URB597). A number of compounds were prepared by copper-catalyzed [3+2] cycloaddition reactions between azides and alkynes (click chemistry). Exploiting the same chemistry, we also synthesized carbamates bearing a substituted (1-benzyltriazol-4-yl)-methyl moiety at both the O and N end. The click chemistry approach allowed us rapidly to explore the structure–activity relationships within the class. Several single-digit nanomolar inhibitors of rat FAAH were obtained, including potent derivative **26a**, which showed an IC_{50} value of 1.4 nM and inhibited brain FAAH activity in vivo. Some of these compounds potently inhibited human FAAH-1. In particular, compounds **34b** and **40**, bearing a [1-[(methoxyphenyl)methyl]triazol-4-yl]methyl group at both the O and N end of the carbamate function, displayed single-digit nanomolar IC_{50} values for both rat and human FAAH. In addition, they showed a remarkable improvement in rat plasma stability and kinetic solubility in buffer with respect to URB524 (**1a**).

The dramatic decrease in FAAH inhibitory activity of 1-phenyl- or 1-benzyl-substituted *O*-(1,2,4-triazol-3-yl)mehtyl carbamates **43** and **46** demonstrated that the 1-substituted-(1,2,3-triazol-4-yl)methyl core structure, easily accessible by click chemistry, was essential for obtaining potent inhibition of FAAH activity.

In conclusion, exploiting a click chemistry approach, we prepared a novel series of potent and drug-like FAAH inhibitors containing an *O*-(1,2,3-triazol-4-yl)alkyl carbamate moiety. The compounds described in the present study represent a promising starting point for the development of new FAAH inhibitors with improved drug-like properties.

Experimental Section

Chemistry

Chemicals, materials and methods: Solvents and reagents were obtained from commercial suppliers and were used without further purification. Automated column chromatography purifications were performed by using a Teledyne ISCO apparatus (CombiFlash R_f) with prepacked silica gel (SiO_2) columns of different sizes (4–120 g). Mixtures of increasing polarity of Cy and EtOAc or CH_2Cl_2

Table 4. Comparison of rat versus human fatty acid amide hydrolase (FAAH)-1 inhibitory activity of selected compounds.

Compd	IC_{50} [nM][a]	
	r-FAAH	*h*-FAAH
1a	5.7 ± 1.2	3.1 ± 1.3
23a	9.9 ± 1.9	619 ± 138
23b	12.1 ± 0.8	180 ± 6
24a	6.2 ± 1.3	1068 ± 144
24b	13.2 ± 2.8	190 ± 8
26a	1.4 ± 0.2	337 ± 101
26b	11.8 ± 3.7	158 ± 12
27	10.5 ± 2.9	3.6 ± 0.9
28	11.9 ± 1.0	812 ± 105
29	10.4 ± 2.6	216 ± 6
34b	3.9 ± 0.7	4.2 ± 1.0
37	3.2 ± 1.2	30.4 ± 3.7
38	7.6 ± 0.6	30.7 ± 6.2
39	9.8 ± 0.4	39.1 ± 0.6
40	5.8 ± 0.6	9.4 ± 2.0

[a] IC_{50} values are the mean ± SEM ($n = 3$).

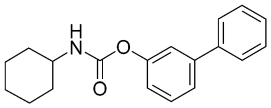
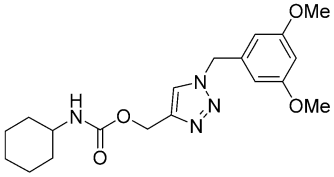
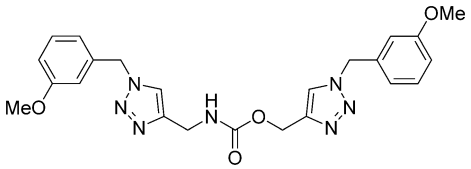
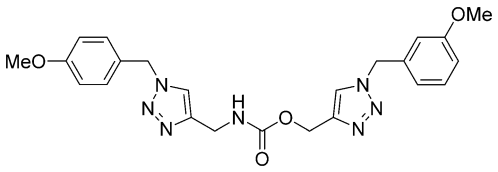
and MeOH were used as eluents. NMR experiments were run on a Bruker Avance III 400 system (400.13 MHz for ^1H; 100.62 MHz for ^{13}C), equipped with a BBI probe and Z-gradients. Spectra were acquired at 300 K, using deuterated dimethylsulfoxide ([D_6]DMSO) or deuterated chloroform ($CDCl_3$) as solvents. Chemical shifts (δ) for ^1H and ^{13}C spectra are reported in parts per million (ppm) relative to the residual nondeuterated solvent ($CDCl_3$: $\delta_H = 7.26$ ppm and $\delta_C = 77.16$ ppm; [D_6]DMSO: $\delta_H = 2.50$ ppm and $\delta_C = 39.52$ ppm). UPLC/MS analyses were run on a Waters ACQUITY UPLC/MS system consisting of a single quadropole detector (SQD) mass spectrometer (MS) equipped with an electrospray ionization (ESI) interface and a PDA detector. The PDA range was 210–400 nm. ESI in both the positive and negative mode was applied. Mobile phases: (A) 10 mM NH_4OAc in H_2O, pH 5; (B) 10 mM NH_4OAc in MeCN/H_2O (95:5), pH 5. Analyses were performed either with method A or B. **Method A**: gradient 5→95% B over 3 min; flow rate 0.5 mL min^{-1}; temperature: 40 °C. Pre-column: Vanguard BEH C_{18} (1.7 μm, 2.1 × 5 mm). Column: BEH C_{18} (1.7 μm, 2.1 × 50 mm). **Method B**: gradient: 50→100% B over 3 min, flow rate 0.5 mL min^{-1}; temperature: 40 °C. Pre-column: Vanguard BEH C_{18} (1.7 μm, 2.1 × 5 mm). Column: BEH C_{18} (1.7 μm, 2.1 × 50 mm). High-resolution mass spectrometry (HRMS) was performed on a Synapt G2 Quadrupole-Tof Instrument (Waters, USA), equipped with an ESI ion source.

All final compounds (**4**, **17**–**30**, **32**, **33**, **34a**,**b**, **37**–**40**, **43** and **46**) showed ≥95% purity by NMR and UPLC/MS analysis. The syntheses of reaction intermediates **3**, **5a**–**c**, **6**–**16**, **31a**--**b**, **35a**–**c**, **36a**–**c**, **41**, **42a**,**b**, and **45** are described in the Supporting Information.

Abbreviations: acetonitrile (CH_3CN), benzyl bromide (BnBr), cyclohexane (Cy), dichloromethane (CH_2Cl_2), diethyl ether (Et_2O), 4-(dimethylamino)-pyridine (DMAP), ethanol (EtOH), ethyl acetate (EtOAc), hydrochloric acid (HCl), methanol (MeOH), *N*,*N*′-dimethylformamide (DMF), room temperature (rt), sodium sulfate (Na_2SO_4), sodium bicarbonate ($NaHCO_3$), sulfuric acid (H_2SO_4), *tert*-butanol (*t*-BuOH), tetrahydrofuran (THF), triethylamine (Et_3N).

General procedure (1) for the synthesis of triazoles 17–30, 37–40: Ethynyl derivative (1 equiv) and azido compound (1 equiv)

Table 5. A comparison of the overall profile of *O*-arylcarbamate **1a** with compounds **27**, **34b**, and **40**.

Compd	Structure	IC_{50} [nM][a]		Plasma stab. [% at 7 h][b]	MLM stab. $t_{\frac{1}{2}}$ [min][c]	Kinetic sol. [μM][d]
		r-FAAH	*h*-FAAH			
1a		5.7 ± 1.2	3.1 ± 1.3	0	< 5 ± 0	6
27		10.5 ± 2.9	3.6 ± 0.9	91 ± 3	5 ± 0	18
34b		3.9 ± 0.7	4.2 ± 1.0	88 ± 11	6 ± 0	174
40		5.8 ± 0.6	9.4 ± 2.0	89 ± 9	8 ± 1	152

[a] IC_{50} values for FAAH inhibition are the mean ± SEM ($n = 3$); [b] Plasma stability; data are the mean ± SEM ($n = 3$); [c] Half-life in mouse liver microsomes (MLM); data are the mean ± SD ($n = 3$); [d] Phosphate-buffered saline (pH 7.4), data represent a single determination.

were suspended in a solution of water/*t*-BuOH (1:1). A freshly prepared 1 M aq solution sodium ascorbate (0.1 equiv) was added, followed by the addition of $CuSO_4 \cdot 5H_2O$ (0.01 equiv). The resulting reaction was vigorously stirred for 3 h at rt. The reaction mixture was then diluted with water, cooled on ice, and the precipitate was collected by filtration. When addition of water failed to precipitate the desired triazole, evaporation of the solvent allowed the recovery of the crude product. Purification was performed by column chromatography.

General procedure (2) for the synthesis of double triazoles carbamates 34a,b: Ethynyl derivative (0.5 equiv) and azido compound (1 equiv) were suspended in a solution of water/*t*-BuOH (1:1). A freshly prepared 1 M aq solution sodium ascorbate (0.1 equiv) was added, followed by the addition of $CuSO_4 \cdot 5H_2O$ (0.01 equiv). The resulting reaction was vigorously stirred for 8 h at rt. Afterwards, evaporation of the solvent allowed the recovery of the crude product. Purification was performed by column chromatography.

(3-Phenylphenyl)methyl *N*-cyclohexylcarbamate (4): (3-Phenylphenyl)methanol (**3**, 0.125 g, 0.68 mmol) was dissolved in dry CH_3CN (5 mL) while stirring at rt. Then, DMAP (0.08 g, 0.68 mmol) and cyclohexyl isocyanate (0.09 g, 0.75 mmol) were added, and the reaction mixture was stirred at 80 °C for 3 h. Afterwards, the reaction mixture was diluted with EtOAc (20 mL) and washed once with 2 N aq HCl (20 mL), and once with brine (20 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated in vacuo. The crude product was purified by chromatography (MeOH/CH_2Cl_2, 0→2%) to afford **4** as a white powder (0.13 g; 61%): ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 7.69–7.61 (m, 3H), 7.60 (dt, J = 7.9, 1.4 Hz, 1H), 7.47 (q, J = 7.3 Hz, 3H), 7.42–7.31 (m, 2H), 7.19 (d, J = 7.9 Hz, 1H), 5.07 (s, 2H), 3.31–3.22 (m, 1H), 1.76 (dd, J = 12.6, 3.6 Hz, 2H), 1.67 (dq, J = 12.5, 3.7 Hz, 2H), 1.53 (dq, J = 11.5, 3.7 Hz, 1H), 1.31–1.00 ppm (m, 5H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 155.2, 140.2, 139.9, 138.0, 128.9, 128.9, 127.5, 126.7, 126.6, 126.0 (2C), 65.0, 49.5, 32.6 (2C), 25.1, 24.6 ppm (2C); UPLC-MS: Method B, t_R = 1.97 min, ionization: *m/z* 310 $[M+H]^+$; HRMS-ESI: *m/z* $[M+Na]^+$ calcd for $C_{20}H_{23}NO_2Na$: 332.1626, found: 332.1622.

(1-Phenyltriazol-4-yl)methyl *N*-cyclohexylcarbamate (17): The reaction was carried out following general procedure (1), using prop-2-ynyl *N*-cyclohexylcarbamate (0.23 g, 1.26 mmol), azidobenzene (0.15 g, 1.26 mmol), sodium ascorbate (0.02, 0.12 mmol), and $CuSO_4 \cdot 5H_2O$ (0.003 g, 0.01 mmol) in water/*t*-BuOH (1:1; 3 mL). Purification was performed by flash chromatography (MeOH/CH_2Cl_2, 0→2%) to afford **17** as a white powder (0.22 g; 59%): ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.82 (s, 1H), 7.91 (m, 2H), 7.61 (m, 2H), 7.52 (m, 1H), 7.20 (d, J = 8.0 Hz, 1H), 5.13 (s, 2H), 3.28 (m, 1H), 1.76 (m, 2H), 1.67 (dt, J = 12.2, 3.8 Hz, 2H), 1.54 (m, 1H), 1.18 ppm (m, 5H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 155.4, 144.3, 137.0, 130.3 (2C), 129.2, 123.2, 120.6 (2C), 57.0, 50.0, 33.1 (2C), 25.6, 25.0 ppm (2C); UPLC-MS: Method A, t_R = 2.42 min, ionization: *m/z* 301 $[M+H]^+$; HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_{16}H_{20}N_4O_2$: 301.1665, found: 301.1666.

(1-Benzyltriazol-4-yl)methyl *N*-cyclohexylcarbamate (18): The reaction was carried out following general procedure (1), using prop-2-ynyl *N*-cyclohexylcarbamate (0.15 g, 0.82 mmol), azidomethylbenzene (0.11 g, 0.82 mmol), sodium ascorbate (0.016, 0.08 mmol), and $CuSO_4 \cdot 5H_2O$ (0.002 g, 0.008 mmol) in water/*t*-BuOH (1:1; 3 mL). Purification was performed by flash chromatography (EtOAc/Cy, 0→50%) to afford **18** as a white powder (0.18 g; 71%): ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.15 (s, 1H), 7.35 (m, 5H), 7.13 (d, J = 7.9 Hz, 1H), 5.60 (s, 2H), 5.02 (s, 2H), 3.25 (m, 1H), 1.69 (m, 4H), 1.53 (dt, J = 12.7, 3.8 Hz, 1H), 1.15 ppm (m, 5H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 155.4, 143.5, 136.5, 129.2 (2C), 128.6, 128.4 (2C), 125.0, 57.2, 53.2, 49.9, 33.0 (2C), 25.6, 25.0 ppm (2C); UPLC-MS: Method A, t_R = 2.37 min, ionization: *m/z* 315 $[M+H]^+$; HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_{17}H_{22}N_4O_2$: 315.1821, found: 315.1826.

(1-Phenethyltriazol-4-yl)methyl *N*-cyclohexylcarbamate (19): The reaction was carried out following general procedure (1), using prop-2-ynyl *N*-cyclohexylcarbamate (0.15 g, 0.82 mmol), 2-azidoethylbenzene (0.12 g, 0.82 mmol), sodium ascorbate (0.016, 0.08 mmol), and $CuSO_4 \cdot 5H_2O$ (0.002 g, 0.008 mmol) in water/*t*-BuOH (1:1; 3 mL). Purification was performed by flash chromatography (MeOH/CH_2Cl_2, 0→2%) to afford **19** as a white powder (0.135 g; 50%): ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.03 (s, 1H), 7.29 (m, 2H), 7.21 (m, 3H), 7.13 (d, J = 7.9 Hz, 1H), 4.99 (s, 2H), 4.61 (dd, J = 7.9, 6.8 Hz, 2H), 3.24 (m, 1H), 3.16 (t, J = 7.4 Hz, 2H), 1.69 (m, 4H), 1.54 (d, J = 13.3 Hz, 1H), 1.18 ppm (m, 5H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 154.6, 142.5, 137.5, 128.6 (2C), 128.3 (2C), 126.5, 124.4, 56.6, 50.3, 49.4, 35.6, 32.6 (2C), 25.1, 24.5 ppm (2C); UPLC-MS: Method A, t_R = 2.44 min, ionization: *m/z* 329 $[M+H]^+$; HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_{18}H_{24}N_4O_2$: 329.1978, found: 329.1982.

(1-Benzhydryltriazol-4-yl)methyl *N*-cyclohexylcarbamate (20): The reaction was carried out following general procedure (1), using prop-2-ynyl *N*-cyclohexylcarbamate (0.15 g, 0.82 mmol), [azido(phenyl)methyl]benzene (0.17 g, 0.82 mmol), sodium ascorbate (0.016, 0.08 mmol), and $CuSO_4 \cdot 5H_2O$ (0.002 g, 0.008 mmol) in water/*t*-BuOH (1:1; 3 mL). Purification was performed by flash chromatography (EtOAc/Cy, 0→40%) to afford **20** as a white powder (0.23 g; 71%): ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.08 (s, 1H), 7.39 (m, 6H), 7.31 (s, 1H), 7.21 (m, 4H), 7.14 (d, J = 7.9 Hz, 1H), 5.02 (s, 2H), 3.22 (m, 1H), 1.67 (m, 4H), 1.52 (d, J = 12.5 Hz, 1H), 1.12 ppm (m, 5H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 155.4, 144.3, 138.6 (2C), 128.7 (4C), 128.2 (2C), 127.9 (4C), 66.5, 56.7, 49.4, 32.5 (2C), 25.1, 24.5 ppm (2C); UPLC-MS: Method A, t_R = 2.81 min, ionization: *m/z* 391 $[M+H]^+$; HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_{23}H_{26}N_4O_2$: 391.2134, found: 391.2132.

[1-(2-Naphthylmethyl)triazol-4-yl]methyl *N*-cyclohexylcarbamate (21): The reaction was carried out following general procedure (1), using prop-2-ynyl *N*-cyclohexylcarbamate (0.15 g, 0.82 mmol), 2-(azidomethyl)naphthalene (0.15 g, 0.82 mmol), sodium ascorbate (0.016, 0.08 mmol), and $CuSO_4 \cdot 5H_2O$ (0.002 g, 0.008 mmol) in water/*t*-BuOH (1:1; 3 mL). Purification was performed by flash chromatography (MeOH/CH_2Cl_2, 0→2%) to afford **21** as a white powder (0.16 g; 54%): ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.19 (s, 1H), 7.92 (m, 3H), 7.86 (s, 1H), 7.54 (m, 2H), 7.44 (dd, J = 8.5, 1.6 Hz, 1H), 7.10 (d, J = 7.8 Hz, 1H), 5.76 (s, 2H), 5.01 (s, 2H), 3.22 (m, 1H), 1.66 (dd, J = 26.8, 12.6 Hz, 4H), 1.51 (d, J = 12.5 Hz, 1H), 1.11 ppm (m, 5H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 154.9, 143.0, 133.4, 132.7, 132.4, 128.4, 127.7, 127.5, 126.9, 126.5, 126.4, 125.7, 124.6, 56.7, 52.9, 49.4, 32.5 (2C), 25.1, 24.5 ppm (2C); UPLC-MS: Method A, t_R = 2.65 min, ionization: *m/z* 365 $[M+H]^+$; HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_{21}H_{24}N_4O_2$: 365.1978, found: 365.1975.

[1-[(2-Cyanophenyl)methyl]triazol-4-yl]methyl *N*-cyclohexylcarbamate (22a): The reaction was carried out following general procedure (1), using prop-2-ynyl *N*-cyclohexylcarbamate (0.15 g, 0.82 mmol), 2-(azidomethyl)benzonitrile (0.13 g, 0.82 mmol), sodium ascorbate (0.016, 0.08 mmol), and $CuSO_4 \cdot 5H_2O$ (0.002 g, 0.008 mmol) in water/*t*-BuOH (1:1; 3 mL). Purification was performed by flash chromatography (MeOH/CH_2Cl_2, 0→2%) to afford **22a** as a white powder (0.15 g; 53%): ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.19 (s, 1H), 7.92 (dd, J = 7.7, 1.0 Hz, 1H), 7.72 (td, J = 7.7, 1.2 Hz, 1H), 7.57 (td, J = 7.7, 0.9 Hz, 1H), 7.36 (d, J = 7.8 Hz,

1H), 7.14 (d, $J=7.8$ Hz, 1H), 5.81 (s, 2H), 5.03 (s, 2H), 3.25 (m, 1H), 1.68 (dd, $J=27.0$, 12.5 Hz, 4H), 1.53 (d, $J=12.7$ Hz, 1H), 1.13 ppm (dtd, $J=31.1$, 24.1, 12.1 Hz, 5H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta=$ 154.9, 143.1, 138.7, 133.8, 133.3, 129.4, 129.2, 125.0, 116.9, 111.2, 56.6, 50.9, 49.4, 32.6 (2C), 25.1, 24.5 ppm (2C); UPLC-MS: Method A, $t_R=2.25$ min, ionization: *m/z* 340 $[M+H]^+$; HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_{18}H_{21}N_5O_2$: 340.1773, found: 340.1779.

[1-[(3-Cyanophenyl)methyl]triazol-4-yl]methyl *N*-cyclohexylcarbamate (22b): The reaction was carried out following general procedure (1), using prop-2-ynyl *N*-cyclohexylcarbamate (0.15 g, 0.82 mmol), 3-(azidomethyl)benzonitrile (0.13 g, 0.82 mmol), sodium ascorbate (0.016, 0.08 mmol), and $CuSO_4{\cdot}5H_2O$ (0.002 g, 0.008 mmol) in water/*t*-BuOH (1:1; 3 mL). Purification was performed by flash chromatography (MeOH/CH_2Cl_2, 0→2%) to afford **22b** as a white powder (0.17 g; 62%): ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=8.21$ (s, 1H), 7.82 (m, 2H), 7.62 (m, 2H), 7.12 (d, $J=$ 7.8 Hz, 1H), 5.67 (s, 2H), 5.01 (s, 2H), 3.24 (m, 1H), 1.68 (dd, $J=$ 25.4, 12.5 Hz, 4H), 1.52 (d, $J=12.6$ Hz, 1H), 1.13 ppm (m, 5H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta=154.9$, 143.2, 137.5, 132.9, 131.9, 131.6, 130.0, 124.8, 118.3, 111.6, 56.6, 51.7, 49.4, 32.6 (2C), 25.1, 24.5 ppm (2C); UPLC-MS: Method A, $t_R=2.25$ min, ionization: *m/z* 340 $[M+H]^+$; HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_{18}H_{21}N_5O_2$: 340.1773, found: 340.1781.

[1-[(4-Cyanophenyl)methyl]triazol-4-yl]methyl *N*-cyclohexylcarbamate (22c): The reaction was carried out following general procedure (1), using prop-2-ynyl *N*-cyclohexylcarbamate (0.15 g, 0.82 mmol), 4-(azidomethyl)benzonitrile (0.13 g, 0.82 mmol), sodium ascorbate (0.016, 0.08 mmol), and $CuSO_4{\cdot}5H_2O$ (0.002 g, 0.008 mmol) in water/*t*-BuOH (1:1; 3 mL). Purification was performed by flash chromatography (MeOH/CH_2Cl_2, 0→2%) to afford **22c** as a white powder (0.21 g; 77%): ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=8.21$ (s, 1H), 7.86 (d, $J=8.2$ Hz, 2H), 7.45 (d, $J=$ 8.2 Hz, 2H), 7.14 (d, $J=7.8$ Hz, 1H), 5.72 (s, 2H), 5.02 (s, 2H), 3.24 (m, 1H), 1.69 (dd, $J=25.1$, 12.6 Hz, 4H), 1.53 (d, $J=12.4$ Hz, 1H), 1.14 ppm (m, 5H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta=154.9$, 143.2, 141.4, 132.7 (2C), 128.6 (2C), 124.9, 118.4, 110.9, 56.6, 52.1, 49.4, 32.6 (2C), 25.1, 24.5 ppm (2C); UPLC-MS: Method A, $t_R=2.22$ min, ionization: *m/z* 340 $[M+H]^+$; HRMS-ESI: *m/z* $[M+Na]^+$ calcd for $C_{18}H_{21}N_5O_2Na$: 362.1593, found: 362.1594.

[1-[(2-Fluorophenyl)methyl]triazol-4-yl]methyl *N*-cyclohexylcarbamate (23a): The reaction was carried out following general procedure (1), using prop-2-ynyl *N*-cyclohexylcarbamate (0.15 g, 0.82 mmol), 1-(azidomethyl)-2-fluorobenzene (0.12 g, 0.82 mmol), sodium ascorbate (0.016, 0.08 mmol), and $CuSO_4{\cdot}5H_2O$ (0.002 g, 0.008 mmol) in water/*t*-BuOH (1:1; 3 mL). Purification was performed by flash chromatography (MeOH/CH_2Cl_2, 0→2%) to afford **23a** as a white powder (0.17 g; 61%): ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=8.11$ (s, 1H), 7.42 (m, 1H), 7.34 (td, $J=7.6$, 1.4 Hz, 1H), 7.23 (m, 2H), 7.13 (d, $J=7.8$ Hz, 1H), 5.66 (s, 2H), 5.00 (s, 2H), 3.24 (m, 1H), 1.67 (m, 4H), 1.52 (d, $J=12.5$ Hz, 1H), 1.12 ppm (m, 5H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta=160.0$ (d, $J=246.7$ Hz), 155.0, 143.0, 130.7 (d, $J=4.6$ Hz), 130.7, 124.8 (d, $J=3.4$ Hz), 124.7, 122.8 (d, $J=14.7$ Hz), 115.6 (d, $J=20.8$ Hz), 56.7, 49.4, 46.8 (d, $J=$ 3.7 Hz), 32.6 (2C), 25.1, 24.5 ppm (2C); UPLC-MS: Method A, $t_R=$ 2.37 min, ionization: *m/z* 333 $[M+H]^+$; HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_{17}H_{21}FN_4O_2$: 333.1727, found: 333.1732.

[1-[(3-Fluorophenyl)methyl]triazol-4-yl]methyl *N*-cyclohexylcarbamate (23b): The reaction was carried out following general procedure (1), using prop-2-ynyl *N*-cyclohexylcarbamate (0.15 g, 0.82 mmol), 1-(azidomethyl)-3-fluorobenzene (0.12 g, 0.82 mmol), sodium ascorbate (0.016, 0.08 mmol), and $CuSO_4{\cdot}5H_2O$ (0.002 g, 0.008 mmol) in water/*t*-BuOH (1:1; 3 mL). Purification was performed by flash chromatography (EtOAc/Cy, 0→50%) to afford **23b** as a white powder (0.17 g; 63%): ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=8.18$ (s, 1H), 7.42 (m, 1H), 7.16 (m, 4H), 5.62 (s, 2H), 5.01 (s, 2H), 3.24 (m, 1H), 1.68 (dd, $J=26.2$, 12.5 Hz, 4H), 1.52 (d, $J=12.5$ Hz, 1H), 1.12 ppm (m, 5H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta=162.5$ (d, $J=244.3$ Hz), 155.4, 143.6, 139.2, 131.3 (d, $J=8.3$ Hz), 125.2, 124.4 (d, $J=2.7$ Hz), 115.3 (m, 2C), 57.1, 52.5, 49.9, 33.0 (2C), 25.6, 25.0 ppm (2C); UPLC-MS: Method A, $t_R=2.40$ min, ionization: *m/z* 333 $[M+H]^+$; HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_{17}H_{21}FN_4O_2$: 333.1727, found: 333.1731.

[1-[(4-Fluorophenyl)methyl]triazol-4-yl]methyl *N*-cyclohexylcarbamate (23c): The reaction was carried out following general procedure (1), using prop-2-ynyl *N*-cyclohexylcarbamate (0.15 g, 0.82 mmol), 1-(azidomethyl)-4-fluorobenzene (0.12 g, 0.82 mmol), sodium ascorbate (0.016, 0.08 mmol), and $CuSO_4{\cdot}5H_2O$ (0.002 g, 0.008 mmol) in water/*t*-BuOH (1:1; 3 mL). Purification was performed by flash chromatography (MeOH/CH_2Cl_2, 0→2%) to afford **23c** as a white powder (0.13 g; 49%): ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=8.14$ (s, 1H), 7.38 (ddd, $J=8.4$, 5.3, 2.5 Hz, 2H), 7.20 (m, 2H), 7.12 (d, $J=7.5$ Hz, 1H), 5.58 (s, 2H), 5.00 (s, 2H), 3.23 (m, 1H), 1.67 (m, 4H), 1.52 (d, $J=12.5$ Hz, 1H), 1.11 ppm (m, 5H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta=161.8$ (d, $J=244.5$ Hz), 154.9, 143.0, 132.2, 130.2 (d, $J=8.4$ Hz), 124.5, 115.5 (d, $J=21.6$ Hz), 56.7, 51.9, 49.4, 32.6 (2C), 25.1, 24.5 ppm (2C); UPLC-MS: Method A, $t_R=$ 2.39 min, ionization: *m/z* 333 $[M+H]^+$; HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_{17}H_{21}FN_4O_2$: 333.1727, found: 333.1731.

[1-[(2-Chlorophenyl)methyl]triazol-4-yl]methyl *N*-cyclohexylcarbamate (24a): The reaction was carried out following general procedure (1), using prop-2-ynyl *N*-cyclohexylcarbamate (0.15 g, 0.82 mmol), 1-(azidomethyl)-2-chlorobenzene (0.14 g, 0.82 mmol), sodium ascorbate (0.016, 0.08 mmol), and $CuSO_4{\cdot}5H_2O$ (0.002 g, 0.008 mmol) in water/*t*-BuOH (1:1; 3 mL). Purification was performed by flash chromatography (MeOH/CH_2Cl_2, 0→2%) to afford **24a** as a white powder (0.19 g; 68%): ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=8.11$ (s, 1H), 7.52 (dd, $J=7.7$, 1.5 Hz, 1H), 7.38 (m, 2H), 7.22 (dd, $J=7.4$, 1.7 Hz, 1H), 7.14 (d, $J=7.7$ Hz, 1H), 5.70 (s, 2H), 5.02 (s, 2H), 3.24 (m, 1H), 1.68 (dd, $J=26.2$, 12.5 Hz, 4H), 1.53 (m, 1H), 1.11 ppm (m, 5H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta=$ 154.9, 142.9, 133.2, 132.6, 130.4, 130.2, 129.6, 127.6, 124.9, 56.7, 50.5, 49.4, 32.6 (2C), 25.1, 24.5 ppm (2C); UPLC-MS: Method A, $t_R=$ 2.49 min, ionization: *m/z* 349 $[M+H]^+$; HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_{17}H_{21}ClN_4O_2$: 349.1431, found: 349.1435.

[1-[(3-Chlorophenyl)methyl]triazol-4-yl]methyl *N*-cyclohexylcarbamate (24b): The reaction was carried out following general procedure (1), using prop-2-ynyl *N*-cyclohexylcarbamate (0.15 g, 0.82 mmol), 1-(azidomethyl)-3-chlorobenzene (0.14 g, 0.82 mmol), sodium ascorbate (0.016, 0.08 mmol), and $CuSO_4{\cdot}5H_2O$ (0.002 g, 0.008 mmol) in water/*t*-BuOH (1:1; 3 mL). Purification was performed by flash chromatography (EtOAc/Cy, 0→50%) to afford **24b** as a white powder (0.24 g; 83%): ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=8.19$ (s, 1H), 7.40 (dd, $J=6.1$, 2.3 Hz, 3H), 7.27 (dq, $J=5.9$, 2.8 Hz, 1H), 7.13 (d, $J=7.9$ Hz, 1H), 5.61 (s, 2H), 5.01 (s, 2H), 3.25 (m, 1H), 1.68 (dd, $J=26.4$, 12.4 Hz, 4H), 1.52 (d, $J=12.5$ Hz, 1H), 1.13 ppm (m, 5H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta=154.9$, 143.1, 138.4, 133.2, 130.6, 128.1, 127.8, 126.6, 124.7, 56.6, 51.9, 49.4, 32.6 (2C), 25.1, 24.5 ppm (2C); UPLC-MS: Method A, $t_R=2.55$ min, ionization: *m/z* 349 $[M+H]^+$; HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_{17}H_{21}ClN_4O_2$: 349.1431, found: 349.1436.

[1-[(4-Chlorophenyl)methyl]triazol-4-yl]methyl *N*-cyclohexylcarbamate (24c): The reaction was carried out following general pro-

cedure (1), using prop-2-ynyl *N*-cyclohexylcarbamate (0.15 g, 0.82 mmol), 1-(azidomethyl)-4-chlorobenzene (0.14 g, 0.82 mmol), sodium ascorbate (0.016, 0.08 mmol), and $CuSO_4{\cdot}5H_2O$ (0.002 g, 0.008 mmol) in water/*t*-BuOH (1:1; 3 mL). Purification was performed by flash chromatography (EtOAc/Cy, 0→50%) to afford **24c** as a white powder (0.13 g; 47%): ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.15 (s, 1H), 7.44 (m, 2H), 7.33 (d, J = 8.5 Hz, 2H), 7.12 (d, J = 7.8 Hz, 1H), 5.60 (s, 2H), 5.00 (s, 2H), 3.23 (m, 1H), 1.67 (m, 4H), 1.52 (m, 1H), 1.11 ppm (m, 5H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 154.9, 143.1, 135.0, 132.8, 129.8 (2C), 128.7 (2C), 124.6, 56.6, 51.9, 49.4, 32.6 (2C), 25.1, 24.5 ppm (2C); UPLC-MS: Method A, t_R = 2.55 min, ionization: *m/z* 349 $[M+H]^+$; HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_{17}H_{21}ClN_4O_2$: 349.1431, found: 349.1427.

[1-(*o*-Tolylmethyl)triazol-4-yl]methyl *N*-cyclohexylcarbamate (25a): The reaction was carried out following general procedure (1), using prop-2-ynyl *N*-cyclohexylcarbamate (0.37 g, 2.04 mmol), 1-(azidomethyl)-2-methylbenzene (0.3 g, 2.04 mmol), sodium ascorbate (0.040, 0.2 mmol), and $CuSO_4{\cdot}5H_2O$ (0.005 g, 0.02 mmol) in water/*t*-BuOH (1:1; 3 mL). Purification was performed by flash chromatography (MeOH/CH_2Cl_2, 0→2%) to afford **25a** as a white powder (0.13 g; 47%): ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.03 (s, 1H), 7.29–7.12 (m, 4H), 7.07 (d, J = 7.5 Hz, 1H), 5.60 (s, 2H), 5.00 (s, 2H), 3.22 (dt, J = 10.7, 5.8 Hz, 1H), 2.30 (s, 3H), 1.82–1.58 (m, 4H), 1.52 (d, J = 12.5 Hz, 1H), 1.31–0.95 ppm (m, 5H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 155.4, 143.3, 136.7, 134.5, 130.8, 129.1, 128.8, 126.7, 125.1, 57.2, 51.3, 49.9, 33.0 (2C), 25.5, 25.0 (2C), 19.0 ppm; UPLC-MS: Method A, t_R = 2.5 min, ionization: *m/z* 329 $[M+H]^+$; HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_{18}H_{24}N_4O_2$: 329.1978, found: 329.1977.

[1-(*m*-Tolylmethyl)triazol-4-yl]methyl *N*-cyclohexylcarbamate (25b): The reaction was carried out following general procedure (1), using prop-2-ynyl *N*-cyclohexylcarbamate (0.37 g, 2.04 mmol), 1-(azidomethyl)-3-methylbenzene (0.3 g, 2.04 mmol), sodium ascorbate (0.040, 0.2 mmol), and $CuSO_4{\cdot}5H_2O$ (0.005 g, 0.02 mmol) in water/*t*-BuOH (1:1; 3 mL). Purification was performed by flash chromatography (MeOH/CH_2Cl_2, 0→2%) to afford **25b** as a white powder (0.17 g; 65%): ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.12 (s, 1H), 7.25 (t, J = 7.8 Hz, 1H), 7.13 (m, 4H), 5.54 (s, 2H), 5.00 (s, 2H), 3.24 (m, 1H), 2.28 (s, 3H), 1.68 (dd, J = 25.9, 12.5 Hz, 4H), 1.52 (d, J = 12.5 Hz, 1H), 1.12 ppm (m, 5H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 154.9, 142.9, 137.9, 135.8, 128.7, 128.6, 128.5, 125.0, 124.5, 56.7, 52.7, 49.4, 32.5 (2C), 25.1, 24.5 (2C), 20.8 ppm; UPLC-MS: Method A, t_R = 2.52 min, ionization: *m/z* 329 $[M+H]^+$; HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_{18}H_{24}N_4O_2$: 329.1978, found: 329.1981.

[1-(*p*-Tolylmethyl)triazol-4-yl]methyl *N*-cyclohexylcarbamate (25c): The reaction was carried out following general procedure (1), using prop-2-ynyl *N*-cyclohexylcarbamate (0.37 g, 2.04 mmol), 1-(azidomethyl)-4-methylbenzene (0.3 g, 2.04 mmol), sodium ascorbate (0.040, 0.2 mmol), and $CuSO_4{\cdot}5H_2O$ (0.005 g, 0.02 mmol) in water/*t*-BuOH (1:1; 3 mL). Purification was performed by flash chromatography (MeOH/CH_2Cl_2, 0→2%) to afford **25c** as a white powder (0.18 g; 67%): ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.10 (s, 1H), 7.28–7.08 (m, 5H), 5.53 (s, 2H), 4.99 (s, 2H), 3.30–3.15 (m, 1H), 2.27 (s, 3H), 1.67 (dd, J = 24.7, 12.5 Hz, 4H), 1.52 (d, J = 12.2 Hz, 1H), 1.15 ppm (dt, J = 37.6, 12.2 Hz, 5H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 155.4, 143.4, 137.9, 133.4, 129.7 (2C), 128.4 (2C), 124.9, 57.2, 53.0, 49.9, 33.0 (2C), 25.5, 25.0 (2C), 21.1 ppm; UPLC-MS: Method A, t_R = 2.52 min, ionization: *m/z* 329 $[M+H]^+$; HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_{18}H_{24}N_4O_2$: 329.1978, found: 329.1978.

[1-[(2-Methoxyphenyl)methyl]triazol-4-yl]methyl *N*-cyclohexylcarbamate (26a): The reaction was carried out following general procedure (1), using prop-2-ynyl *N*-cyclohexylcarbamate (0.15 g, 0.82 mmol), 1-(azidomethyl)-2-methoxybenzene (0.13 g, 0.82 mmol), sodium ascorbate (0.016, 0.08 mmol), and $CuSO_4{\cdot}5H_2O$ (0.002 g, 0.008 mmol) in water/*t*-BuOH (1:1; 3 mL). Purification was performed by flash chromatography (MeOH/CH_2Cl_2, 0→2%) to afford **26a** as a white powder (0.18 g; 64%): ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 7.98 (s, 1H), 7.35 (m, 1H), 7.09 (m, 3H), 6.93 (m, 1H), 5.52 (s, 2H), 4.99 (s, 2H), 3.82 (s, 3H), 3.23 (m, 1H), 1.67 (m, 4H), 1.52 (d, J = 12.6 Hz, 1H), 1.12 ppm (m, 5H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 156.8, 155.0, 142.7, 130.0, 129.6, 124.6, 123.5, 120.5, 111.2, 56.7, 55.5, 49.4, 48.2, 32.6 (2C), 25.1, 24.5 ppm (2C); UPLC-MS: Method A, t_R = 2.43 min, ionization: *m/z* 345 $[M+H]^+$; HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_{18}H_{24}N_4O_3$: 345.1927, found: 345.1930.

[1-[(3-Methoxyphenyl)methyl]triazol-4-yl]methyl *N*-cyclohexylcarbamate (26b): The reaction was carried out following general procedure (1), using prop-2-ynyl *N*-cyclohexylcarbamate (0.15 g, 0.82 mmol), 1-(azidomethyl)-3-methoxybenzene (0.13 g, 0.82 mmol), sodium ascorbate (0.016, 0.08 mmol), and $CuSO_4{\cdot}5H_2O$ (0.002 g, 0.008 mmol) in water/*t*-BuOH (1:1; 3 mL). Purification was performed by flash chromatography (EtOAc/Cy, 0→70%) to afford **26b** as a white powder (0.14 g; 50%): ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.14 (s, 1H), 7.28 (td, J = 7.5, 1.8 Hz, 1H), 7.12 (d, J = 7.8 Hz, 1H), 6.90 (d, J = 6.2 Hz, 2H), 6.85 (d, J = 7.8 Hz, 1H), 5.55 (s, 2H), 5.00 (s, 2H), 3.73 (s, 3H), 3.24 (d, J = 7.6 Hz, 1H), 1.68 (dd, J = 25.7, 12.5 Hz, 4H), 1.52 (d, J = 12.4 Hz, 1H), 1.13 ppm (m, 5H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 159.4, 154.9, 143.0, 137.4, 129.8, 124.6, 119.9, 113.7, 113.4, 56.7, 55.0, 52.6, 49.4, 32.6 (2C), 25.1, 24.5 ppm (2C); UPLC-MS: Method A, t_R = 2.38 min, ionization: *m/z* 345 $[M+H]^+$; HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_{18}H_{24}N_4O_3$: 345.1927, found: 345.1929.

[1-[(4-Methoxyphenyl)methyl]triazol-4-yl]methyl *N*-cyclohexylcarbamate (26c): The reaction was carried out following general procedure (1), using prop-2-ynyl *N*-cyclohexylcarbamate (0.15 g, 0.82 mmol), 1-(azidomethyl)-4-methoxybenzene (0.13 g, 0.82 mmol), sodium ascorbate (0.016, 0.08 mmol), and $CuSO_4{\cdot}5H_2O$ (0.002 g, 0.008 mmol) in water/*t*-BuOH (1:1; 3 mL). Purification was performed by flash chromatography (EtOAc/Cy, 0→60%) to afford **26c** as a white powder (0.15 g; 54%): ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.08 (s, 1H), 7.29 (d, J = 8.6 Hz, 2H), 7.11 (d, J = 7.8 Hz, 1H), 6.92 (d, J = 8.6 Hz, 2H), 5.50 (s, 2H), 4.99 (s, 2H), 3.73 (s, 3H), 3.22 (m, 1H), 1.68 (dd, J = 25.3, 12.6 Hz, 4H), 1.52 (d, J = 12.6 Hz, 1H), 1.13 ppm (m, 5H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 159.1, 154.9, 142.9, 129.5 (2C), 127.9, 124.2, 114.0 (2C), 56.7, 55.1, 52.2, 49.4, 32.6 (2C), 25.1, 24.5 ppm (2C); UPLC-MS: Method A, t_R = 2.35 min, ionization: *m/z* 345 $[M+H]^+$; HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_{18}H_{24}N_4O_3$: 345.1927, found: 345.1924.

[1-[(3,5-Dimethoxyphenyl)methyl]triazol-4-yl]methyl *N*-cyclohexylcarbamate (27): The reaction was carried out following general procedure (1), using prop-2-ynyl *N*-cyclohexylcarbamate (0.15 g, 0.82 mmol), 1-(azidomethyl)-3,5-dimethoxybenzene (0.16 g, 0.82 mmol), sodium ascorbate (0.016, 0.08 mmol), and $CuSO_4{\cdot}5H_2O$ (0.002 g, 0.008 mmol) in water/*t*-BuOH (1:1; 3 mL). Purification was performed by flash chromatography (EtOAc/Cy, 0→40%) to afford **27** as a white powder (0.17 g; 55%): ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.14 (s, 1H), 7.12 (d, J = 7.8 Hz, 1H), 6.46 (s, 3H), 5.50 (s, 2H), 5.01 (s, 2H), 3.72 (s, 6H), 3.24 (m, 1H), 1.67 (m, 4H), 1.52 (d, J = 12.4 Hz, 1H), 1.12 ppm (m, 5H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 160.6, 154.9, 143.0, 138.0, 124.6, 106.0, 99.5, 56.7, 55.2, 52.7, 49.4, 32.6 (2C), 25.1, 24.5 ppm (2C); UPLC-MS: Method A, t_R = 2.42 min, ionization: *m/z* 375 $[M+H]^+$; HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_{19}H_{26}N_4O_4$: 375.2032, found: 375.2047.

[1-[(2,6-Difluorophenyl)methyl]triazol-4-yl]methyl *N*-cyclohexylcarbamate (28): The reaction was carried out following general procedure (1), using prop-2-ynyl *N*-cyclohexylcarbamate (0.15 g, 0.82 mmol), 2-(azidomethyl)-1,3-difluorobenzene (0.14 g, 0.82 mmol), sodium ascorbate (0.016, 0.08 mmol), and $CuSO_4{\cdot}5H_2O$ (0.002 g, 0.008 mmol) in water/*t*-BuOH (1:1; 3 mL). Purification was performed by flash chromatography (EtOAc/Cy, 0→40%) to afford **28** as a white powder (0.16 g; 56%): ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ=8.10 (s, 1H), 7.51 (m, 1H), 7.18 (t, J=8.1 Hz, 2H), 7.13 (d, J=7.8 Hz, 1H), 5.66 (s, 2H), 4.99 (s, 2H), 3.23 (m, 1H), 1.68 (dd, J=26.0, 12.6 Hz, 4H), 1.52 (d, J=12.6 Hz, 1H), 1.12 ppm (m, 5H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ=161.2 (d, J=249.2 Hz), 155.4, 143.3, 132.1 (t, J=10.4 Hz), 125.1, 112.3 (d, J=24.4 Hz), 111.7 (t, J=19.3 Hz), 57.1, 49.9, 41.2 (t, J=3.7 Hz), 33.0 (2C), 25.6, 25.0 ppm (2C); UPLC-MS: Method A, t_R=2.38 min, ionization: *m/z* 351 $[M+H]^+$; HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_{17}H_{20}F_2N_4O_2$: 351.1633, found: 351.1631.

[1-[(3,5-Difluorophenyl)methyl]triazol-4-yl]methyl *N*-cyclohexylcarbamate (29): The reaction was carried out following general procedure (1), using prop-2-ynyl *N*-cyclohexylcarbamate (0.15 g, 0.82 mmol), 1-(azidomethyl)-3,5-difluorobenzene (0.14 g, 0.82 mmol), sodium ascorbate (0.016, 0.08 mmol), and $CuSO_4{\cdot}5H_2O$ (0.002 g, 0.008 mmol) in water/*t*-BuOH (1:1; 3 mL). Purification was performed by flash chromatography (MeOH/CH_2Cl_2, 0→2%) to afford **29** as a white powder (0.22 g; 77%): ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ=8.21 (s, 1H), 7.23 (tt, J=9.4, 2.3 Hz, 1H), 7.13 (d, J=7.8 Hz, 1H), 7.04 (t, J=6.4 Hz, 2H), 5.64 (s, 2H), 5.02 (s, 2H), 3.23 (m, 1H), 1.68 (dd, J=26.4, 12.4 Hz, 4H), 1.52 (d, J=12.6 Hz, 1H), 1.13 ppm (m, 5H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ=162.8 (dd, J=247.1, 13.2 Hz), 155.4, 143.7, 140.6 (t, J=9.4 Hz), 125.4, 111.7 (m), 104.1 (t, J=25.7 Hz), 57.1, 52.1, 49.9, 33.0 (2C), 25.5, 25.0 ppm (2C); UPLC-MS: Method A, t_R=2.46 min, ionization: *m/z* 351 $[M+H]^+$; HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_{17}H_{20}F_2N_4O_2$: 351.1633, found: 351.1634.

[1-[(2-Fluoro-3-methoxy-phenyl)methyl]triazol-4-yl]methyl *N*-cyclohexylcarbamate (30): The reaction was carried out following general procedure (1), using prop-2-ynyl *N*-cyclohexylcarbamate (0.15 g, 0.82 mmol), 1-(azidomethyl)-2-fluoro-3-methoxybenzene (0.15 g, 0.82 mmol), sodium ascorbate (0.016, 0.08 mmol), and $CuSO_4{\cdot}5H_2O$ (0.002 g, 0.008 mmol) in water/*t*-BuOH (1:1; 3 mL). Purification was performed by flash chromatography (MeOH/CH_2Cl_2, 0→2%) to afford **30** as a white powder (0.23 g; 77%): ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ=8.09 (s, 1H), 7.14 (m, 3H), 6.84 (m, 1H), 5.64 (s, 2H), 5.00 (s, 2H), 3.83 (s, 3H), 3.24 (m, 1H), 1.68 (dd, J=25.9, 12.5 Hz, 4H), 1.52 (d, J=12.5 Hz, 1H), 1.12 ppm (m, 5H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ=154.9, 149.5 (d, J=246.8 Hz), 147.3 (d, J=10.0 Hz), 142.9, 124.7, 124.6 (d, J=4.6 Hz), 123.5 (d, J=11.9 Hz), 121.1 (d, J=2.0 Hz), 114.0 (d, J=1.3 Hz), 56.7, 56.1, 49.4, 46.7 (d, J=4.6 Hz), 32.6 (2C), 25.1, 24.5 ppm (2C); UPLC-MS: Method A, t_R=2.37 min, ionization: *m/z* 363 $[M+H]^+$; HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_{18}H_{23}FN_4O_3$: 363.1832, found: 363.1834.

2-(1-Phenyltriazol-4-yl)ethyl *N*-cyclohexylcarbamate (32): 2-(1-Phenyltriazol-4-yl)ethanol (**31a**, 0.24 g, 1.26 mmol) was dissolved in dry CH_3CN (5 mL) under stirring. Then, DMAP (0.15 g, 1.26 mmol) and cyclohexyl isocyanate (0.17 g, 1.38 mmol) were added, and the reaction mixture was stirred overnight at 80 °C. The mixture was then diluted with EtOAc (30 mL) and washed once with 2 N aq HCl (30 mL), and once with brine (30 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated in vacuo. Purification was performed by flash chromatography (MeOH/CH_2Cl_2, 0→2%) to afford **31a** as a white powder (0.25 g; 63%): ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ=8.62 (s, 1H), 7.87 (m, 2H), 7.60 (m, 2H), 7.49 (m, 1H), 7.06 (d, J=8.0 Hz, 1H), 4.25 (t, J=6.7 Hz, 2H), 3.24 (m, 1H), 3.02 (t, J=6.7 Hz, 2H), 1.69 (m, 4H), 1.53 (d, J=13.3 Hz, 1H), 1.15 ppm (m, 5H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ=155.7, 145.1, 137.2, 130.3 (2C), 128.9, 121.4, 120.3 (2C), 62.7, 49.8, 33.1 (2C), 26.0, 25.6, 25.0 ppm (2C); UPLC-MS: Method A, t_R=2.39 min, ionization: *m/z* 315 $[M+H]^+$; HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_{17}H_{22}N_4O_2$: 315.1821, found: 315.1829.

2-(1-Benzyltriazol-4-yl)ethyl N-cyclohexylcarbamate (33): The reaction was carried out according to the procedure employed for **32**, starting from 2-(1-benzyltriazol-4-yl)ethanol (**31b**, 0.23 g, 1.12 mmol), cyclohexyl isocyanate (0.15 g, 1.23 mmol), and DMAP (0.14 g, 1.12 mmol) in dry CH_3CN (5 mL). Purification was performed by flash chromatography (MeOH/CH_2Cl_2, 0→2%) to afford **33** as a white powder (0.2 g; 54%): ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ=7.95 (s, 1H), 7.33 (m, 5H), 7.01 (d, J=7.96 Hz, 1H), 5.55 (s, 2H), 4.16 (t, J=6.83 Hz, 2H), 3.21 (m, 1H), 2.90 (t, J=6.82 Hz, 2H), 1.67 (m, 4H), 1.52 (m, 1H), 1.12 ppm (m, 5H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ=155.0, 144.1, 136.6, 129.1 (2C), 128.5, 128.3 (2C), 123.1, 99.9, 62.8, 53.1, 49.8, 33.1 (2C), 26.0, 25.6, 25.0 ppm (2C); UPLC-MS: Method A, t_R=2.33 min, ionization: *m/z* 329 $[M+H]^+$; HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_{18}H_{24}N_4O_2$: 329.1978, found: 329.1983.

[1-[(2-Methoxyphenyl)methyl]triazol-4-yl]methyl *N*-[[1-[(2-methoxyphenyl)methyl]triazol-4-yl]methyl]carbamate (34a): The reaction was carried out following general procedure (2), using prop-2-ynyl *N*-prop-2-ynylcarbamate (0.11 g, 0.767 mmol), 1-(azidomethyl)-2-methoxybenzene (0.25 g, 1.53 mmol), sodium ascorbate (0.030, 0.15 mmol), and $CuSO_4{\cdot}5H_2O$ (0.004 g, 0.002 mmol) in water/*t*-BuOH (1:1; 3 mL). Purification was performed by flash chromatography (MeOH/CH_2Cl_2, 0→4%) to afford **34a** as a white amorphous solid (0.2 g; 58%): ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ=8.00 (s, 1H), 7.80 (s, 1H), 7.68 (t, J=5.7 Hz, 1H), 7.34 (m, 2H), 7.08 (m, 4H), 6.93 (m, 2H), 5.51 (s, 2H), 5.48 (s, 2H), 5.03 (s, 2H), 4.21 (d, J=5.8 Hz, 2H), 3.81 (s, 3H), 3.80 ppm (s, 3H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ=156.83, 156.8, 155.9, 145.0, 142.5, 130.0, 129.9, 129.6, 129.5, 124.7, 123.6, 123.4, 122.8, 120.5 (2C), 111.2, 111.1, 57.1, 55.5 (2C), 48.2, 48.1, 35.9 ppm; UPLC-MS: Method A, t_R=2.18 min, ionization: *m/z* 464 $[M+H]^+$; HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_{23}H_{25}N_7O_4$: 464.2046, found: 464.2056.

[1-[(3-Methoxyphenyl)methyl]triazol-4-yl]methyl *N*-[[1-[(3-methoxyphenyl)methyl]triazol-4-yl]methyl]carbamate (34b): The reaction was carried out following general procedure (2), using prop-2-ynyl N-prop-2-ynylcarbamate (0.11 g, 0.767 mmol), 1-(azidomethyl)-3-methoxybenzene (0.25 g, 1.53 mmol), sodium ascorbate (0.030, 0.15 mmol), and $CuSO_4{\cdot}5H_2O$ (0.004 g, 0.002 mmol) in water/*t*-BuOH (1:1; 3 mL). Purification was performed by flash chromatography (MeOH/CH_2Cl_2, 0→5%) to afford **34b** as a white amorphous solid (0.29 g; 81%): ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ=8.15 (s, 1H), 7.95 (s, 1H), 7.71 (t, J=5.7 Hz, 1H), 7.28 (td, J=8.2, 7.5, 2.1 Hz, 2H), 6.89 (dd, J=5.8, 2.9 Hz, 4H), 6.85 (d, J=7.3 Hz, 2H), 5.55 (s, 2H), 5.51 (s, 2H), 5.04 (s, 2H), 4.22 (d, J=5.8 Hz, 2H), 3.73 ppm (s, 6H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ=159.4 (2C), 155.9, 145.3, 142.8, 137.5, 137.4, 129.9, 129.8, 124.6, 122.8, 120.0 (2C), 113.7, 113.7, 113.4, 113.4, 57.1, 55.0 (2C), 52.7, 52.6, 36.0 ppm; UPLC-MS: Method A, t_R=2.12 min, ionization: *m/z* 464 $[M+H]^+$; HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_{23}H_{25}N_7O_4$: 464.2046, found: 464.2052.

[1-[(2-Methoxyphenyl)methyl]triazol-4-yl]methyl *N*-[[1-[(3-methoxyphenyl)methyl]triazol-4-yl]methyl]carbamate (37): The reaction was carried out following general procedure (1), using prop-2-ynyl *N*-[[1-[(3-methoxyphenyl)methyl]triazol-4-yl]methyl]carbamate (**36b**, 0.15 g, 0.5 mmol), 1-(azidomethyl)-2-methoxybenzene

(0.08 g, 0.5 mmol), sodium ascorbate (0.010, 0.05 mmol), and $CuSO_4 \cdot 5H_2O$ (0.001 g, 0.005 mmol) in water/*t*-BuOH (1:1; 3 mL). Purification was performed by flash chromatography (MeOH/CH_2Cl_2, 0→2%) to afford **37** as a white amorphous solid (0.13 g; 58%): ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.00 (s, 1H), 7.95 (s, 1H), 7.70 (t, J = 5.7 Hz, 1H), 7.34 (m, 1H), 7.27 (m, 1H), 7.11 (dd, J = 7.5, 1.4 Hz, 1H), 7.05 (d, J = 8.2 Hz, 1H), 6.92 (m, 3H), 6.85 (d, J = 7.6 Hz, 1H), 5.51 (s, 4H), 5.03 (s, 2H), 4.21 (d, J = 5.8 Hz, 2H), 3.80 (s, 3H), 3.73 ppm (s, 3H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 159.8, 157.3, 156.4, 145.8, 142.9, 137.9, 130.4, 130.3, 130.1, 125.2, 123.9, 123.3, 120.9, 120.4, 114.2, 113.8, 111.6, 57.6, 56.0, 55.5, 53.1, 48.7, 36.4 ppm; UPLC-MS: Method A, t_R = 2.15 min, ionization: *m/z* 464 $[M+H]^+$; HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_{23}H_{25}N_7O_4$: 464.2046, found: 464.2062.

[1-[(2-Methoxyphenyl)methyl]triazol-4-yl]methyl *N*-[[1-[(4-methoxyphenyl)methyl]triazol-4-yl]methyl]carbamate (38): The reaction was carried out following general procedure (1), using prop-2-ynyl *N*-[[1-[(4-methoxyphenyl)methyl]triazol-4-yl]methyl]carbamate (**36c**, 0.15 g, 0.5 mmol), 1-(azidomethyl)-2-methoxybenzene (0.08 g, 0.5 mmol), sodium ascorbate (0.010, 0.05 mmol), and $CuSO_4 \cdot 5H_2O$ (0.001 g, 0.005 mmol) in water/*t*-BuOH (1:1; 3 mL). Purification was performed by flash chromatography (MeOH/CH_2Cl_2, 0→2%) to afford **38** as a colorless amorphous solid (0.15 g; 63%): ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.00 (s, 1H), 7.89 (s, 1H), 7.68 (t, J = 5.7 Hz, 1H), 7.34 (m, 1H), 7.28 (d, J = 8.5 Hz, 2H), 7.11 (dd, J = 7.4, 1.3 Hz, 1H), 7.05 (d, J = 8.2 Hz, 1H), 6.92 (m, 3H), 5.51 (s, 2H), 5.46 (s, 2H), 5.03 (s, 2H), 4.20 (d, J = 5.8 Hz, 2H), 3.80 (s, 3H), 3.73 ppm (s, 3H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 159.5, 157.3, 156.3, 145.8, 142.9, 130.4, 130.1, 130.0 (2C), 128.4, 125.2, 123.9, 122.9, 120.9, 114.5 (2C), 111.7, 57.6, 56.0, 55.5, 52.7, 48.7, 36.4 ppm; UPLC-MS: Method A, t_R = 2.13 min, ionization: *m/z* 464 $[M+H]^+$; HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_{23}H_{25}N_7O_4$: 464.2046, found: 464.2052.

[1-[(3-Methoxyphenyl)methyl]triazol-4-yl]methyl *N*-[[1-[(2-methoxyphenyl)methyl]triazol-4-yl]methyl]carbamate (39): The reaction was carried out following general procedure (1), using prop-2-ynyl *N*-[[1-[(2-methoxyphenyl)methyl]triazol-4-yl]methyl]carbamate (**36a**, 0.15 g, 0.5 mmol), 1-(azidomethyl)-3-methoxybenzene (0.08 g, 0.5 mmol), sodium ascorbate (0.010, 0.05 mmol), and $CuSO_4 \cdot 5H_2O$ (0.001 g, 0.005 mmol) in water/*t*-BuOH (1:1; 3 mL). Purification was performed by flash chromatography (MeOH/CH_2Cl_2, 0→2%) to afford **39** as a colorless amorphous solid (0.15 g; 66%): ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.15 (s, 1H), 7.80 (s, 1H), 7.69 (t, J = 5.7 Hz, 1H), 7.34 (t, J = 7.8 Hz, 1H), 7.28 (m, 1H), 7.06 (dd, J = 14.4, 7.8 Hz, 2H), 6.89 (m, 4H), 5.54 (s, 2H), 5.48 (s, 2H), 5.04 (s, 2H), 4.21 (d, J = 5.8 Hz, 2H), 3.80 (s, 3H), 3.73 ppm (s, 3H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 159.4, 156.8, 155.9, 145.0, 142.8, 137.4, 129.9 (2C), 129.5, 124.6, 123.6, 122.8, 120.5, 120.0, 113.7, 113.4, 111.1, 57.1, 55.5, 55.0, 52.7, 48.1, 35.9 ppm; UPLC-MS: Method A, t_R = 2.15 min, ionization: *m/z* 464 $[M+H]^+$; HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_{23}H_{25}N_7O_4$: 464.2046, found: 464.2044.

[1-[(3-Methoxyphenyl)methyl]triazol-4-yl]methyl *N*-[[1-[(4-methoxyphenyl)methyl]triazol-4-yl]methyl]carbamate (40): The reaction was carried out following general procedure (1), using prop-2-ynyl *N*-[[1-[(4-methoxyphenyl)methyl]triazol-4-yl]methyl]carbamate (**36c**, 0.15 g, 0.5 mmol), 1-(azidomethyl)-3-methoxybenzene (0.08 g, 0.5 mmol), sodium ascorbate (0.010, 0.05 mmol), and $CuSO_4 \cdot 5H_2O$ (0.001 g, 0.005 mmol) in water/*t*-BuOH (1:1; 3 mL). Purification was performed by flash chromatography (MeOH/CH_2Cl_2, 0→2%) to afford **40** as a colorless amorphous solid (0.16 g; 68%): ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.15 (s, 1H), 7.89 (s, 1H), 7.69 (t, J = 5.7 Hz, 1H), 7.28 (m, 3H), 6.90 (m, 4H), 6.85 (d, J = 7.7 Hz, 1H), 5.55 (s, 2H), 5.46 (s, 2H), 5.04 (s, 2H), 4.20 (d, J = 5.8 Hz, 2H), 3.73 ppm (s, 6H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 159.4, 159.1, 155.9, 145.3, 142.8, 137.4, 129.9, 129.6 (2C), 127.9, 124.6, 122.4, 120.0, 114.0 (2C), 113.7, 113.4, 57.1, 55.1, 55.0, 52.6, 52.2, 35.9 ppm; UPLC-MS: Method A, t_R = 2.09 min, ionization: *m/z* 464 $[M+H]^+$; HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_{23}H_{25}N_7O_4$: 464.2046, found: 464.2052.

(1-(1-Phenyl-1,2,4-triazol-3-yl)methyl *N*-cyclohexylcarbamate (43): The reaction was carried out following the procedure employed for **32**, starting from (1-phenyl-1,2,4-triazol-3-yl)methanol (**42a**, 0.05 g, 0.28 mmol), cyclohexyl isocyanate (0.04 g, 0.30 mmol), and DMAP (0.03 g, 0.28 mmol) in dry CH_3CN (5 mL). Purification was performed by flash chromatography (EtOAc/Cy, 0→100%) to afford **43** as a white powder (0.05 g; 58%): ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 9.26 (s, 1H), 7.89–7.77 (m, 2H), 7.62–7.49 (m, 2H), 7.48–7.37 (m, 1H), 7.26 (d, J = 7.8 Hz, 1H), 5.08 (s, 2H), 3.26 (ddd, J = 10.5, 7.2, 3.2 Hz, 1H), 1.86–1.60 (m, 4H), 1.53 (d, J = 12.8 Hz, 1H), 1.30–1.01 ppm (m, 5H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 161.0, 155.2, 143.5, 137.0, 130.2, 128.3, 119.7, 58.8, 50.0, 33.1 (2C), 25.6, 25.0 ppm (2C); UPLC-MS: Method A, t_R = 2.34 min, ionization: *m/z* 301 $[M+H]^+$; HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_{16}H_{20}N_4O_2$: 301.1665, found: 301.1674.

Benzyl-1,2,4-triazol-3-yl)methyl *N*-cyclohexylcarbamate (46): The reaction was carried out following the procedure employed for **32**, starting from (1-benzyl-1,2,4-triazol-3-yl)methanol (**42b**, 0.06 g, 0.32 mmol), cyclohexyl isocyanate (0.044 g, 0.35 mmol), and DMAP (0.04 g, 0.32 mmol) in dry CH_3CN (5 mL). Purification was performed by flash chromatography (EtOAc/Cy, 0→100%) to afford **46** as a white powder (0.07 g; 68%): ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.62 (s, 1H), 7.42–7.25 (m, 5H), 7.17 (d, J = 7.7 Hz, 1H), 5.37 (s, 2H), 4.93 (s, 2H), 3.27–3.15 (m, 1H), 1.68 (dd, J = 28.7, 12.5 Hz, 4H), 1.52 (d, J = 12.6 Hz, 1H), 1.13 ppm (tdd, J = 32.8, 24.1, 12.1 Hz, 5H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 159.7, 154.7, 145.0, 136.1, 128.6, 127.9, 127.9, 58.4, 52.1, 49.4, 32.5 (2C), 25.1, 24.5 ppm (2C); UPLC-MS: Method A, t_R = 2.27 min, ionization: *m/z* 315 $[M+H]^+$; HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_{17}H_{22}N_4O_2$: 315.1821, found: 315.1823.

Pharmacology

Animals: CD1 male mice were obtained from Charles River Laboratories Italia. All procedures were performed in accordance with the Ethical Guidelines of the International Association for the Study of Pain, Italian regulations on the protection of animals used for experimental and other scientific purposes (D.M. 116192) and European Economic Community regulations (O.J. of E.C. L 358/1 12/18/1986). When appropriate, procedures were also approved by the Institutional Animal Care and Use Committee of the University of California, Irvine (USA).

In vitro rat FAAH radiometric assay: Rat FAAH was prepared from male Sprague–Dawley rat brains, homogenized in a potter in 20 mM of Tris HCl (pH 7.4), 0.32 M sucrose. The radiometric assay used to measure FAAH activity was performed in Eppendorf tubes: total rat brain homogenate (50 μg) were pre-incubated in 445.5 μL of assay buffer (50 mM Tris-HCl pH 7.4, 0.05% fatty-acid-free bovine serum albumin (BSA), Sigma–Aldrich) with 4.5 μL of inhibitor (at appropriate concentration in DMSO) or DMSO alone (to measure FAAH total activity) for 10 min at 37 °C. The blank (no activity control) was prepared using 445.5 μL of assay buffer and 4.5 μL of DMSO without the total rat brain homogenate (50 μg). After 10 min of pre-incubation with test compound, the reaction was started by adding substrate (50 μL) and incubating for 30 min at 37 °C. The substrate was prepared in assay buffer in order to achieve the final concentration of 1 μM arachidonoyl ethanolamide (Cayman Chemical No. 90050) and 0.6 nM anandamide [ethanola-

mine-1-^{3}H] (American Radiolabeled Chemicals Inc., ART. 0626, conc. 1 mCi mL^{-1}, S.A. 60 Ci mmol^{-1}). The reaction was stopped by adding cold $CHCl_3$/MeOH (1:1). After centrifugation (845 *g* at 4 °C for 10 min), 600 μL of aqueous phase were transferred into scintillation vials previously filled with 3 mL of scintillation fluid (Ultima GoldTM, PerkinElmer Inc., Cat. 6013329). Radioactivity was measured by liquid scintillation counting (MicroBeta2 LumiJET PerkinElmer Inc.).

In vitro human FAAH fluorescent assay: Human recombinant FAAH was obtained from a HEK-293 cell line stably overexpressing human FAAH-1 enzyme. Cells were grown in Dulbecco's modified Eagle medium (DMEM) containing 10% fetal bovine serum, 1% penicillin/streptomycin, 1% glutamine, and 500 μg mL^{-1} G418. To obtain membrane preparation, cells were scraped off with cold phosphate-buffered saline (PBS) and collected by centrifugation (500 *g* at 4 °C for 10 min); the cell pellet was re-suspended in 20 mM Tris-HCl pH 7.4, 0.32 M sucrose, disrupted by sonication (10 pulses, 5 times) and centrifuged (800 *g* at 4 °C for 15 min); the collected supernatant was centrifuged at 105 000 *g* for 1 h at 4 °C, and the pellet was re-suspended in PBS. The fluorescent assay to measure FAAH activity was performed in 96-well black plates: human FAAH-1 membrane preparation (2.5 μg) were pre-incubated for 50 min at 37 °C, in 180 μL of assay buffer (50 mM Tris-HCl pH 7.4, 0.05% fatty-acid-free BSA) with inhibitor (10 μL) or DMSO (10 μL) to measure FAAH total activity. The background (no activity) samples were prepared using assay buffer (180 μL) without human FAAH-1 and DMSO (10 μL). The reaction was then started by addition of 10 μL of the substrate (, Cayman Chemical, No. 10005098) dissolved in ethanol and used at a final concentration of 2 μM. The reaction was carried out for 30 min at 37 °C, and fluorescence was measured with a Tecan Infinite M200 nanoquant plate reader (excitation wavelength 350 nm/emission wavelength 460 nm). The concentration causing half-maximal inhibition (IC_{50}) was determined by nonlinear regression analysis of the Log [concentration]/response curves generated with mean replicate values using a four-parameter Hill equation curve fitting with GraphPad Prism 5 (GraphPad Software Inc., CA, USA).

Ex vivo FAAH inhibition assay: CD1 male mice were treated intraperitoneally (i.p.) with test compound (3 mg kg^{-1}) or vehicle (1:1:8, PEG400, Tween® 80 and Saline 0.9%). After treatment for 1 h, the animals were killed by decapitation, and the brain and liver were collected. Samples were homogenized in 1.5 mL of 20 mM Tris-HCl buffer pH 7.4, containing 0.32 M sucrose, and the homogenates were centrifuged at 1000 *g* for 10 min at 4 °C. The supernatants were collected, and the protein concentration was measured by Bradford method (Bio Rad Protein assay kit). FAAH activity was measured using 50 μg of total brain or liver homogenate in 450 μL of assay buffer (50 mM Tris-HCl pH 7.4, 0.05% fatty-acid-free BSA); the blank (no activity sample) was prepared with 450 μL of assay buffer. The reaction was started by adding 50 μL of substrate for 30 min at 37 °C. The substrate was prepared in assay buffer in order to obtain a final concentration of 1 μM arachidonoyl-ethanolamide (Cayman Chemical, No. 90050) and 0.6 nM anandamide [ethanolamine-1-^{3}H] (American Radiolabeled Chemicals Inc., ART.0626, 1 mCi mL^{-1}, specific activity 60 Ci mmoL^{-1}). The reaction was stopped by adding cold $CHCl_3$/MeOH (1:1). After centrifugation (845 *g* at 4 °C for 10 min), 600 μL of the aqueous phase was transferred into scintillation vials previously filled with 3 mL of scintillation fluid (ULTIMA GOLD, Cat. 6013329, PerkinElmer). Radioactivity was measured by liquid scintillation counting (Microbeta2 Lumijet, PerkinElmer Inc.).

Monoacylglycerol lipase (MGL) activity assay: Purified rat MGL (10 ng) was pre-incubated with appropriate test compound for 10 min at 37 °C in 50 mM Tris-HCl, pH 8.0, containing 0.5 mg mL^{-1} fatty-acid-free BSA. The final concentration of vehicle (1% DMSO) had no effect on MGL activity. Then, 2-oleoylglycerol (2-OG, 10 μM final concentration) was added, and the mixture was incubated for additional 30 min at 37 °C. Reactions were stopped by adding $CHCl_3$/MeOH (2:1 *v/v*), containing heptadecanoic acid (5 nmol/sample) as an internal standard. After centrifugation at 2000 *g* at 4 °C for 10 min, the organic layers were collected and dried under a stream of N_2. The lipid extracts were then suspended in $CHCl_3$/MeOH (1:3 *v/v*) and analyzed by liquid chromatography/mass spectrometry (LC/MS).

In vitro rat plasma stability assay: Compounds were diluted in rat plasma with 10% DMSO to help solubilization. Plasma was already pre-heated at 37 °C (30 min). The final compound concentration was 1.0 μM. At appropriate time points (immediately after dilution (0), 30, 60, 120, 240, 360 and 420 min), a 40 μL aliquot of the incubation solution was diluted in 150 μL of cold CH_3CN spiked with 200 nM warfarin as an internal standard. After vortexing for 30 s, the solution was centrifuged at 3500 *g* for 15 min at 4 °C, and the supernatant was transferred for LC/MS analysis on a Waters ACQUITY UPLC/MS TQD system consisting of a triple quadrupole detector (TQD) mass spectrometer equipped with an ESI interface. Briefly, an aliquot of the supernatant (3.0 μL) was injected on a reverse-phase HPLC column (BEH C_{18} 1.7 μm, 2.1×50 mm) and separated with a linear gradient of acetonitrile. Compounds were quantified on the basis of their multiple reaction monitoring (MRM) peak areas. The response factors, calculated on the basis of the internal standard peak area, were then plotted over time. For each compound, analyses were conducted in triplicate; the compound remaining (%) at 420 min with corresponding standard deviation is reported.

In vitro mouse liver microsomes (MLM) stability assay: Compounds were pre-incubated with microsomes in 100 mM TRIS-HCl (pH 7.4) for 15 min. At time zero, cofactors were added. The final incubation conditions for each sample were: 1.25 mg mL^{-1} liver microsomes, 5 μM compound (final DMSO 0.1%), NADP 1 mM, G6P 20 mM, $MgCl_2$ 2 mM, G6P dehydrogenase 2 U. The mixture was kept at 37 °C under shaking. Aliquots (30 μL) were taken at various time points (typically 0, 5, 15, 30, and 60 min) and crashed with 200 μL of acetonitrile spiked with 200 nM warfarin (internal standard). A reference incubation, with microsomes but without cofactors, was kept at 37 °C and sampled at the end of the time course. After vortexing (5 RCF, 3 min) and centrifugation (3300 RCF, 15 min), an aliquot of supernatant (3 μL) was analyzed by LC-MS/MS by MRM.

Aqueous kinetic solubility assay: The kinetic solubility in PBS at pH 7.4 was determined starting from a 10 mM DMSO solution of the test compound. The study was performed by incubation of an aliquot (10 μL) of 10 mM DMSO solution in PBS (pH 7.4) at 25 °C for 24 h, under shaking, followed by centrifugation (21100 RCF, 30 min) and quantification of dissolved compound in the supernatant by UPLC/MS. The compound target concentration in the solution was 250 μM, resulting in a final DMSO concentration of 2.5%. The supernatant was analyzed by UPLC/MS, and the quantification of the dissolved compound was determined by monitoring the UV trace at 215 nm. The kinetic solubility (μM) was calculated by dividing the peak area of the test compound in the supernatant by the peak area of a reference solution (250 μM) of the test compound in CH_3CN/H_2O (1:1), and further multiplied by the concentration of the test compound reference and the dilution factor. The UPLC/MS

analyses were performed on a Waters ACQUITY UPLC/MS system consisting of a SQD mass spectrometer equipped with an ESI interface and a PDA detector. The PDA range was 210–400 nm. The analyses were run on an ACQUITY UPLC BEH C_{18} column (50×2.1 mm ID, particle size 1.7 μm) with a VanGuard BEH C_{18} pre-column (5×2.1 mm ID, particle size 1.7 μm). The mobile phase was 10 mM NH_4OAc in H_2O at pH 5 adjusted with AcOH (A) and 10 mM NH_4OAc in CH_3CN/H_2O (95:5) at pH 5 (B). ESI in the positive and negative mode was applied in the mass scan range 100–500 Da.

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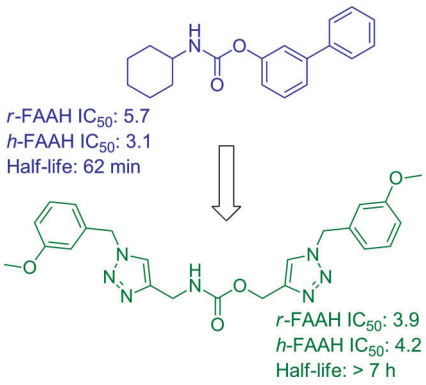
Searching FAAH and wide: *O*-(Triazolyl)methyl carbamates were designed and synthesized via click chemistry to investigate their inhibitory activity against fatty acid amide hydrolase (FAAH), a validated target in the treatment of several disorders, including pain and drug addiction. The results highlighted the importance of 1,4-disubstituted-1,2,3-triazoles for activity.



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_O_-(Triazolyl)methyl Carbamates as a Novel and Potent Class of Fatty Acid Amide Hydrolase (FAAH) Inhibitors