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O-(Triazolyl)methyl carbamates as a novel and potent class of FAAH inhibitors

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Abstract

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. O-aryl carbamates are one of the most representative families. In the search for novel FAAH inhibitors, we synthesized a series of O-(1,2,3-triazol-4-yl)methyl carbamate derivatives exploiting the copper-catalyzed [3 + 2] cycloaddition reaction between azides and alkynes (click chemistry). We explored structure-activity relationships within this new class of compounds and identified potent inhibitors of both rat and human FAAH with IC_{50} values in the single-digit nanomolar range.

Keywords

O-(Triazolyl)methyl carbamates; FAAH; inhibitors; stability; structure-activity relationship

Introduction

Fatty acid amide hydrolase (FAAH)[1] is a membrane-bound serine hydrolase that catalyses 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

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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 heterocycles-based inhibitors,[11] which inhibit FAAH by reversible hemiketal formation with the catalytic serine of the enzyme.[12] Among them, the *O*-arylcarbamate series, exemplified by URB524[8a, 8b, 13] (**1a**, Figure 1), URB597[8a, 8b, 13] (**1b**, Figure 1) and URB694[8c, 14] (**1c**, Figure 1), has been extensively investigated.[15] In particular, it was shown that compound **1b** exerts a combination of anxiolytic-like, antidepressant-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 Ser 241, leading to the formation of carbamoylated, catalytically inactive FAAH and releasing the *O*-biphenyl moiety as the leaving group.

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. The compound 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 worse leaving group than a phenol. Although carbamate-based FAAH inhibitors containing an *O*-(heteroaryl)alkyl moiety have been reported in the patent literature,[12] no *O*-(triazol-4-yl)alkyl carbamate derivatives have been described to date.

We report here 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

The (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).

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.

The 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 the 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).

The *O*-(1,2,3-triazol-4-yl)methyl carbamate derivatives **20**-**30** (Scheme 2) were prepared by reaction of the 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**.

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 the commercially available 1-phenyl-1,2,4-triazole-3-carboxylic acid **41** followed by reaction with cyclohexyl isocyanate. Then, the commercially available methyl *1H*-1,2,4-triazole-3-carboxylate **44** was reacted with benzyl bromide in 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), allowed to recover 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 position 1 and 4 of the triazole ring for FAAH inhibition. The results are reported in Table 1.

The replacement of the phenyl group at position 1 of the triazole with a benzyl residue, compound **18** (IC_{50} = 26 nM), led to a ca. 15-fold increase in potency. It is interesting to note that this compound was only 4.6-fold less potent than **1a** (URB524). 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 a ca. 3-fold decrease in potency.

We then asked whether 1-phenyl- or 1-benzyl-(1,2,4-triazol-3yl)methyl moieties worked as a replacement of the *O*-(3-phenylphenyl) residue of **1a** and whether they were 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, were much less potent than **17** and **18**. We speculate that the loss in potency might be the 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 unfavourable interaction with the active site of the enzyme.

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, the *O*-(1,2,3-triazol-4-yl)methyl carbamates **17** and **18** showed a significantly higher plasma stability than did **1a**. The latter compound displayed half-life of 62 min[26], but was no longer detectable after 7 hours incubation with rat plasma. In contrast, ca. 90% of the initial amount of compound **17** and **18** was still detectable after 7 hours.

The limited decrease in potency of compound **18** vs. **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 were 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 *ortho* or *meta* position all led to low-nanomolar inhibitors. Among them, the *ortho*-methoxybenzyl derivative **26a** showed the highest potency (IC_{50} = 1.4 nM). All the *para*-substituted derivatives were less potent than the corresponding *ortho*- or *meta*-substituted analogues, irrespective of the electronic properties of the susbtituent. In particular, compound **22c** (IC_{50} = 2282 nM), bearing a *para*-cyano group, showed the highest loss in potency within this sub-set 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 fluorine or a methoxyl group at position *ortho* and *meta* led us to synthesize the di-substituted compounds **27**-**30** to verify whether any additive effect on potency was observed. With the exception of the 2-fluoro-3-methoxy-derivative **30** (IC_{50} = 44.6 nM), all the compounds retained an excellent potency, with IC_{50} 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, intraperitoneally) to CD1 mice, FAAH activity measured *ex vivo* in brain tissue was reduced by 78% (n=3) with respect to 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.

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 vs. **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 a ca. 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} 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 FAAH-1 (*h*-FAAH-1). A comparison between the inhibitory potency of selected compounds on *r*-FAAH vs. *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 represented by **27** (IC_{50} = 3.6 nM), which showed 3-fold higher potency on *h*-FAAH-1 with respect to *r*-FAAH. Moreover, the *ortho*-substituted analogues **23a**, **24a**, and **26a**, which displayed single-digit nanomolar *r*-FAAH inhibition, and the 2,6-dibustituted derivative **28**, suffered the most marked loss of potency: 62, 172, 240, and 73-fold respectively. The *meta*-substituted analogues **23b**, **24b**, and **26b**, and the 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 4 to 10-fold lower potency, but retained double-digit nanomolar potency on *h*-FAAH-1. The most promising molecules in terms of potency were **34b** and **40** that possessed IC_{50} for *h*-FAAH-1 inhibition of 4.2 nM and 9.4 nM respectively. Interestingly, both of them are characterized 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, *i.e.* **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). The selective inhibition of FAAH activity by compounds **27**, **34b**, and **40** is in agreement with previous observations with the *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 the *O*-arylcarbamate **1a**. However, they displayed much higher rat plasma stability than **1a**, as ca. 90% of the initial amount of the compounds was still present after 7 hours incubation with rat plasma. Moreover, with the exception of compound **27**, the kinetic solubility in buffer also improved significantly.

Conclusion

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 the 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.

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. For simplicity, solvents and reagents were indicated as follows: acetonitrile (CH_3CN), benzyl bromide (BnBr), cyclohexane (Cy), dichloromethane (DCM), 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).

Automated column chromatography purifications were performed by using a Teledyne ISCO apparatus (CombiFlash® Rf) with pre-packed silica gel columns of different sizes (from 4 g to 120 g). Mixtures of increasing polarity of Cy and EtOAc or DCM and MeOH were used as eluents. NMR experiments were run on a Bruker Avance III 400 system (400.13 MHz for ^{1}H, and 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 for ^{1}H and ^{13}C spectra were recorded in parts per million using the residual non-deuterated solvent as the internal standard (for $CDCl_3$: 7.26 ppm, ^{1}H and 77.16 ppm, ^{13}C; for $[D_6]$DMSO: 2.50 ppm, ^{1}H; 39.52 ppm, ^{13}C). 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 Photodiode Array (PDA) Detector. PDA range was 210-400 nm. ESI in positive and negative mode was applied. Mobile phases: (A) 10mM NH_4OAc in H_2O, pH 5; (B) 10mM NH_4OAc in $MeCN/H_2O$ (95:5) pH 5. Analyses were performed either with method A or B. *Method A*: gradient 5 to 95% B over 3 min; flow rate 0.5 mL/min; temperature 40 °C. Pre column: Vanguard BEH C_{18} (1.7μm 2.1x5mm). Column: BEH C18 (1.7μm 2.1x50mm). *Method B:* gradient: 50 to 100% B over 3 min, flow rate 0.5 mL/min; temperature 40 °C. Pre column: Vanguard BEH C_{18} (1.7μm 2.1x5mm). Column: BEH C18 (1.7μm 2.1x50mm). Accurate mass measurement (HMRS) 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.

General procedure (1) for the synthesis of triazoles (17-30, 37-40)

1 equiv. of the ethynyl derivatives and 1 equiv. of the azido compounds were suspended in a solution of water / *t*-BuOH 1:1. Sodium ascorbate (0.1 eq) of a freshly prepared 1 M solution in water was added, followed by the addition of copper (II) sulfate pentahydrate (0.01 eq). The resulting reaction 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 triazoles, evaporation of the solvent allowed the recovery of the crude products. Purifications were performed by column chromatography.

General procedure (2) for the synthesis of double triazoles carbamates (34a-b)

0.5 equiv. of the ethynyl derivatives and 1 equiv. of the azido compounds were suspended in a solution of water / *t*-BuOH 1:1. Sodium ascorbate (0.1 eq) of a freshly prepared 1 M solution in water was added, followed by the addition of copper (II) sulfate pentahydrate (0.01 eq). The resulting reaction vigorously stirred for 8 h rt. Afterwards, evaporation of the solvent allowed the recovery of the crude products. Purification were performed by column chromatography.

(3-phenylphenyl)methyl N-cyclohexylcarbamate (4)

(3-phenylphenyl)methanol (**3**, 0.125 g, 0.68 mmol) were 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 and washed once with 2N HCl, and once with brine. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by chromatography employing a gradient of MeOH in DCM from 0% to 2%, to afford compound **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, Rt 1.97, 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) and azidobenzene (0.15 g, 1.26 mmol), sodium ascorbate (0.02, 0.12 mmol), and copper (II) sulfate pentahydrate (0.003 g, 0.01 mmol) in water / *t*-BuOH 1:1 (3 mL). Purification was performed by flash chromatography (SiO_2) eluting with a gradient from 0 to 2% MeOH in DCM, to afford compound **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 (2-C), 129.2, 123.2, 120.6 (2-C), 57.0, 50.0, 33.1 (2-C), 25.6, 25.0 ppm (2-C); UPLC-MS: Method A, Rt 2.42, 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) and azidomethylbenzene (0.11 g, 0.82 mmol), sodium ascorbate (0.016, 0.08 mmol), and copper (II) sulfate pentahydrate (0.002 g, 0.008 mmol) in water / *t*-BuOH 1:1 (3 mL). Purification was performed by flash chromatography (SiO_2) eluting with a gradient from 0 to 50% EtOAc in Cy, to afford compound **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); 13C NMR (100 MHz, [D_6]DMSO): δ=155.4, 143.5, 136.5, 129.2 (2-C), 128.6, 128.4 (2-C), 125.0, 57.2, 53.2, 49.9, 33.0 (2-C), 25.6, 25.0 ppm (2-C); UPLC-MS: Method A, Rt 2.37, 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) and 2-azidoethylbenzene (0.12 g, 0.82 mmol), sodium ascorbate (0.016, 0.08 mmol), and copper (II) sulfate pentahydrate (0.002 g, 0.008 mmol) in water / *t*-BuOH 1:1 (3 mL). Purification was performed by flash chromatography (SiO_2) eluting with a gradient from 0 to 2% MeOH in DCM, to afford compound **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 (2-C), 128.3 (2-C), 126.5, 124.4, 56.6, 50.3, 49.4, 35.6, 32.6 (2-C), 25.1, 24.5 ppm (2-C); UPLC-MS: Method A, Rt 2.44, 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) and [azido(phenyl)methyl]benzene (0.17 g, 0.82 mmol), sodium ascorbate (0.016, 0.08 mmol), and copper (II) sulfate pentahydrate (0.002 g, 0.008 mmol) in water / *t*-BuOH 1:1 (3 mL). Purification was performed by flash chromatography (SiO_2) eluting with a gradient from 0 to 40% EtOAc in Cy, to afford compound **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 (2-C), 128.7 (4-C), 128.2 (2-C), 127.9 (4-C), 66.5, 56.7, 49.4, 32.5 (2-C), 25.1, 24.5 ppm (2-C); UPLC-MS: Method A, Rt 2.81, 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) and 2-(azidomethyl)naphthalene (0.15 g, 0.82 mmol), sodium ascorbate (0.016, 0.08 mmol), and copper (II) sulfate pentahydrate (0.002 g, 0.008 mmol) in water / *t*-BuOH 1:1 (3 mL). Purification was performed by flash chromatography (SiO_2) eluting with a gradient from 0 to 2% MeOH in DCM, to afford compound **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 (2-C), 25.1, 24.5 ppm (2-C); UPLC-MS: Method A, Rt 2.65, 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) and 2-(azidomethyl)benzonitrile (0.13 g, 0.82

mmol), sodium ascorbate (0.016, 0.08 mmol), and copper (II) sulfate pentahydrate (0.002 g, 0.008 mmol) in water / *t*-BuOH 1:1 (3 mL). Purification was performed by flash chromatography (SiO_2) eluting with a gradient from 0 to 2% MeOH in DCM, to afford compound **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): δ=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 (2-C), 25.1, 24.5 ppm (2-C); UPLC-MS: Method A, Rt 2.25, 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) and 3-(azidomethyl)benzonitrile (0.13 g, 0.82 mmol), sodium ascorbate (0.016, 0.08 mmol), and copper (II) sulfate pentahydrate (0.002 g, 0.008 mmol) in water / *t*-BuOH 1:1 (3 mL). Purification was performed by flash chromatography (SiO_2) eluting with a gradient from 0 to 2% MeOH in DCM, to afford compound **22b** as a white powder (0.17 g; 62%): ^{1}H NMR (400 MHz, [D_6]DMSO): δ=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): δ=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 (2-C), 25.1, 24.5 ppm (2-C); UPLC-MS: Method A, Rt 2.25, 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) and 4-(azidomethyl)benzonitrile (0.13 g, 0.82 mmol), sodium ascorbate (0.016, 0.08 mmol), and copper (II) sulfate pentahydrate (0.002 g, 0.008 mmol) in water / *t*-BuOH 1:1 (3 mL). Purification was performed by flash chromatography (SiO_2) eluting with a gradient from 0 to 2% MeOH in DCM, to afford compound **22c** as a white powder (0.21 g; 77%): ^{1}H NMR (400 MHz, [D_6]DMSO): δ=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): δ=154.9, 143.2, 141.4, 132.7 (2-C), 128.6 (2-C), 124.9, 118.4, 110.9, 56.6, 52.1, 49.4, 32.6 (2-C), 25.1, 24.5 ppm (2-C); UPLC-MS: Method A, Rt 2.22, 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) and 1-(azidomethyl)-2-fluoro-benzene (0.12 g, 0.82 mmol), sodium ascorbate (0.016, 0.08 mmol), and copper (II) sulfate pentahydrate

(0.002 g, 0.008 mmol) in water / *t*-BuOH 1:1 (3 mL). Purification was performed by flash chromatography (SiO_2) eluting with a gradient from 0 to 2% MeOH in DCM, to afford compound **23a** as a white powder (0.17 g; 61%): ^{1}H NMR (400 MHz, [D_6]DMSO): δ=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): δ=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 (2-C), 25.1, 24.5 ppm (2-C); UPLC-MS: Method A, Rt 2.37, 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) and 1-(azidomethyl)-3-fluoro-benzene (0.12 g, 0.82 mmol), sodium ascorbate (0.016, 0.08 mmol), and copper (II) sulfate pentahydrate (0.002 g, 0.008 mmol) in water / *t*-BuOH 1:1 (3 mL). Purification was performed by flash chromatography (SiO_2) eluting with a gradient from 0 to 50% EtOAc in Cy, to afford compound **23b** as a white powder (0.17 g; 63%): White powder; yield 63%; ^{1}H NMR (400 MHz, [D_6]DMSO): δ=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): δ=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, 2-C), 57.1, 52.5, 49.9, 33.0 (2-C), 25.6, 25.0 ppm (2-C); UPLC-MS: Method A, Rt 2.40, 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) and 1-(azidomethyl)-4-fluoro-benzene (0.12 g, 0.82 mmol), sodium ascorbate (0.016, 0.08 mmol), and copper (II) sulfate pentahydrate (0.002 g, 0.008 mmol) in water / *t*-BuOH 1:1 (3 mL). Purification was performed by flash chromatography (SiO_2) eluting with a gradient from 0 to 2% MeOH in DCM, to afford compound **23c** as a white powder (0.13 g; 49%): ^{1}H NMR (400 MHz, [D_6]DMSO): δ=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): δ=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 (2-C), 25.1, 24.5 ppm (2-C); UPLC-MS: Method A, Rt 2.39, 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) and 1-(azidomethyl)-2-chloro-benzene (0.14 g, 0.82 mmol), sodium ascorbate (0.016, 0.08 mmol), and copper (II) sulfate pentahydrate (0.002 g, 0.008 mmol) in water / *t*-BuOH 1:1 (3 mL). Purification was performed by flash

chromatography (SiO_2) eluting with a gradient from 0 to 2% MeOH in DCM, to afford compound **24a** as a white powder (0.19 g; 68%): ^{1}H NMR (400 MHz, [D_6]DMSO): δ=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): δ=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 (2-C), 25.1, 24.5 ppm (2-C); UPLC-MS: Method A, Rt 2.49, 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) and 1-(azidomethyl)-3-chloro-benzene (0.14 g, 0.82 mmol), sodium ascorbate (0.016, 0.08 mmol), and copper (II) sulfate pentahydrate (0.002 g, 0.008 mmol) in water / *t*-BuOH 1:1 (3 mL). Purification was performed by flash chromatography (SiO_2) eluting with a gradient from 0 to 50% EtOAc in Cy, to afford compound **24b** as a white powder (0.24 g; 83%): ^{1}H NMR (400 MHz, [D_6]DMSO): δ=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): δ=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 (2-C), 25.1, 24.5 ppm (2-C); UPLC-MS: Method A, Rt 2.55, 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 procedure (1), using prop-2-ynyl *N*-cyclohexylcarbamate (0.15 g, 0.82 mmol) and 1-(azidomethyl)-4-chloro-benzene (0.14 g, 0.82 mmol), sodium ascorbate (0.016, 0.08 mmol), and copper (II) sulfate pentahydrate (0.002 g, 0.008 mmol) in water / *t*-BuOH 1:1 (3 mL). Purification was performed by flash chromatography (SiO_2) eluting with a gradient from 0 to 50% EtOAc in Cy, to afford compound **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 (2-C), 128.7 (2-C), 124.6, 56.6, 51.9, 49.4, 32.6 (2-C), 25.1, 24.5 ppm (2-C); UPLC-MS: Method A, Rt 2.55, 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) and 1-(azidomethyl)-2-methyl-benzene (0.3 g, 2.04 mmol), sodium ascorbate (0.040, 0.2 mmol), and copper (II) sulfate pentahydrate (0.005 g, 0.02 mmol) in water / *t*-BuOH 1:1 (3 mL). Purification was performed by flash chromatography (SiO_2) eluting with a gradient from 0 to 2% MeOH in DCM, to afford compound **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 (2-C), 25.5, 25.0 (2-C), 19.0 ppm; UPLC-MS: Method A, Rt 2.5, 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) and 1-(azidomethyl)-3-methyl-benzene (0.3 g, 2.04 mmol), sodium ascorbate (0.040, 0.2 mmol), and copper (II) sulfate pentahydrate (0.005 g, 0.02 mmol) in water / *t*-BuOH 1:1 (3 mL). Purification was performed by flash chromatography (SiO_2) eluting with a gradient from 0 to 2% MeOH in DCM, to afford compound **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 (2-C), 25.1, 24.5 (2-C), 20.8 ppm; UPLC-MS: Method A, Rt 2.52, 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) and 1-(azidomethyl)-4-methyl-benzene (0.3 g, 2.04 mmol), sodium ascorbate (0.040, 0.2 mmol), and copper (II) sulfate pentahydrate (0.005 g, 0.02 mmol) in water / *t*-BuOH 1:1 (3 mL). Purification was performed by flash chromatography (SiO_2) eluting with a gradient from 0 to 2% MeOH in DCM, to afford compound **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 (2-C), 128.4 (2-C), 124.9, 57.2, 53.0, 49.9, 33.0 (2-C), 25.5, 25.0 (2-C), 21.1 ppm; UPLC-MS: Method A, Rt 2.52, 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) and 1-(azidomethyl)-2-methoxy-benzene (0.13 g, 0.82 mmol), sodium ascorbate (0.016, 0.08 mmol), and copper (II) sulfate pentahydrate (0.002 g, 0.008 mmol) in water / *t*-BuOH 1:1 (3 mL). Purification was performed by flash chromatography (SiO_2) eluting with a gradient from 0 to 2% MeOH in DCM, to afford compound **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 (2-C), 25.1, 24.5 ppm (2-C); UPLC-MS: Method A, Rt 2.43, 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) and 1-(azidomethyl)-3-methoxy-benzene (0.13 g, 0.82 mmol), sodium ascorbate (0.016, 0.08 mmol), and copper (II) sulfate pentahydrate (0.002 g, 0.008 mmol) in water / *t*-BuOH 1:1 (3 mL). Purification was performed by flash chromatography (SiO_2) eluting with a gradient from 0 to 70% EtOAc in Cy, to afford compound **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 (2-C), 25.1, 24.5 ppm (2-C); UPLC-MS: Method A, Rt 2.38, 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) and 1-(azidomethyl)-4-methoxy-benzene (0.13 g, 0.82 mmol), sodium ascorbate (0.016, 0.08 mmol), and copper (II) sulfate pentahydrate (0.002 g, 0.008 mmol) in water / *t*-BuOH 1:1 (3 mL). Purification was performed by flash chromatography (SiO_2) eluting with a gradient from 0 to 60% EtOAc in Cy, to afford compound **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 (2-C), 127.9, 124.2, 114.0 (2-C), 56.7, 55.1, 52.2, 49.4, 32.6 (2-C), 25.1, 24.5 ppm (2-C); UPLC-MS: Method A, Rt 2.35, 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) and 1-(azidomethyl)-3,5-dimethoxy-benzene (0.16 g, 0.82 mmol), sodium ascorbate (0.016, 0.08 mmol), and copper (II) sulfate pentahydrate (0.002 g, 0.008 mmol) in water / *t*-BuOH 1:1 (3 mL). Purification was performed by flash chromatography (SiO_2) eluting with a gradient from 0 to 40% EtOAc in Cy, to afford compound **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 (2-C), 25.1, 24.5 ppm (2-C); UPLC-MS: Method A, Rt 2.42, 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) and 2-(azidomethyl)-1,3-difluoro-benzene (0.14 g, 0.82 mmol), sodium ascorbate (0.016, 0.08 mmol), and copper (II) sulfate pentahydrate (0.002 g, 0.008 mmol) in water / *t*-BuOH 1:1 (3 mL). Purification was performed by flash chromatography (SiO_2) eluting with a gradient from 0 to 40% EtOAc in Cy, to afford compound **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 (2-C), 25.6, 25.0 ppm (2-C); UPLC-MS: Method A, Rt 2.38, 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) and 1-(azidomethyl)-3,5-difluoro-benzene (0.14 g, 0.82 mmol), sodium ascorbate (0.016, 0.08 mmol), and copper (II) sulfate pentahydrate (0.002 g, 0.008 mmol) in water / *t*-BuOH 1:1 (3 mL). Purification was performed by flash chromatography (SiO_2) eluting with a gradient from 0 to 2% MeOH in DCM, to afford compound **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 (2-C), 25.5, 25.0 ppm (2-C); UPLC-MS: Method A, Rt 2.46, 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) and 1-(azidomethyl)-2-fluoro-3-methoxy-benzene (0.15 g, 0.82 mmol), sodium ascorbate (0.016, 0.08 mmol), and copper (II) sulfate pentahydrate (0.002 g, 0.008 mmol) in water / *t*-BuOH 1:1 (3 mL). Purification was performed by flash chromatography (SiO_2) eluting with a gradient from 0 to 2% MeOH in DCM, to afford compound **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 (2-C), 25.1, 24.5 ppm (2-C); UPLC-MS: Method A, Rt 2.37, 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 and washed once with 2N HCl, and once with brine. The organic layer was dried over sodium sulfate and concentrated *in vacuo*. The crude product was purified by by flash chromatography (SiO2) eluting with a gradient from 0 to 2% MeOH in DCM. Purification was performed by flash chromatography (SiO_2) eluting with a gradient from 0 to 2% MeOH in DCM, to afford compound **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 (2-C), 128.9, 121.4, 120.3 (2-C), 62.7, 49.8, 33.1 (2-C), 26.0, 25.6, 25.0 ppm (2-C); UPLC-MS: Method A, Rt 2.39, 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)

It was synthesized 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 (SiO_2) eluting with a gradient from 0 to 2% MeOH in DCM, to afford compound **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 (2-C), 128.5, 128.3 (2-C), 123.1, 99.9, 62.8, 53.1, 49.8, 33.1 (2-C), 26.0, 25.6, 25.0 ppm (2-C); UPLC-MS: Method A, Rt 2.33, 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) and 1-(azidomethyl)-2-methoxy-benzene (0.25 g, 1.53 mmol), sodium ascorbate (0.030, 0.15 mmol), and copper (II) sulfate pentahydrate (0.004 g, 0.002 mmol) in water / *t*-BuOH 1:1 (3 mL). Purification was performed by flash chromatography (SiO_2) eluting with a gradient from 0 to 4% MeOH in DCM, to afford compound **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, Rt 2.18, 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) and 1-(azidomethyl)-3-methoxy-benzene (0.25 g, 1.53 mmol), sodium ascorbate (0.030, 0.15 mmol), and copper (II) sulfate pentahydrate (0.004 g, 0.002 mmol) in water / *t*-BuOH 1:1 (3 mL). Purification was performed by flash chromatography (SiO_2) eluting with a gradient from 0 to 5% MeOH in DCM, to afford compound **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, Rt 2.12, 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) and 1-(azidomethyl)-2-methoxy-benzene (0.08 g, 0.5 mmol), sodium ascorbate (0.010, 0.05 mmol), and copper (II) sulfate pentahydrate (0.001 g, 0.005 mmol) in water / *t*-BuOH 1:1 (3 mL). Purification was performed by flash chromatography (SiO_2) eluting with a gradient from 0 to 2% MeOH in DCM, to afford compound **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, Rt 2.15, 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) with 1-(azidomethyl)-2-methoxy-benzene (0.08 g, 0.5 mmol), sodium ascorbate (0.010, 0.05 mmol), and copper (II) sulfate pentahydrate (0.001 g, 0.005 mmol) in water / *t*-BuOH 1:1 (3 mL). Purification was performed by flash chromatography (SiO_2) eluting with a gradient from 0 to 2% MeOH in DCM, to afford compound **38** as a colourless 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, Rt 2.13, 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) and 1-(azidomethyl)-3-methoxy-benzene (0.08 g, 0.5 mmol), sodium ascorbate (0.010, 0.05 mmol), and copper (II) sulfate pentahydrate (0.001 g, 0.005 mmol) in water / *t*-BuOH 1:1 (3 mL). Purification was performed by flash chromatography (SiO_2) eluting with a gradient from 0 to 2% MeOH in DCM, to afford compound **39** as a colourless 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 (2-C), 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, Rt 2.15, 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) with 1-(azidomethyl)-3-methoxy-benzene (0.08 g, 0.5 mmol), sodium ascorbate (0.010, 0.05 mmol), and copper (II) sulfate pentahydrate (0.001 g, 0.005 mmol) in water / *t*-BuOH 1:1 (3 mL). Purification was performed by flash chromatography (SiO_2) eluting with a gradient from 0 to 2% MeOH in DCM, to afford compound **40** as a colourless 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, Rt 2.09, 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)

It was synthesized according to 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 (SiO_2) eluting with a gradient from 0 to 100% EtOAc in Cy, to afford compound **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, Rt 2.34, 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)

It was synthesized according to 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 (SiO_2) eluting with a gradient from 0 to 100% EtOAc in Cy, to afford compound **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, Rt 2.27, 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

***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: 50 μg of total rat brain homogenate 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)) 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 50 μg of total rat brain homogenate. After 10 min of pre-incubation with test compounds, the reaction was started by adding 50 μL of substrate 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 N. 90050) and 0.6nM anandamide [ethanolamine-1-^{3}H] (American Radiolabeled Chemicals Inc., ART. 0626, conc. 1 mCi/mL, S.A. 60 Ci/mmol). The reaction was stopped by adding cold 1:1 $CHCl_3$/methanol. After 10 min of centrifugation (845xg at 4 °C) 600 μL of aqueous phase were transferred into scintillation vials previously filled with 3mL of scintillation fluid (Ultima GoldTM, Perkin Elmer Inc., Cat. 6013329). Radioactivity was measured by liquid scintillation counting (MicroBeta2 LumiJET Perkin Elmer 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% FBS, 1% pen/strep, 1% glutamine and 500 μg /mL G418. To obtain membrane preparation cells were scraped off with cold PBS and collected by centrifugation (500xg, 10 minutes, 4°C); the cell pellet was re-suspended in 20mM Tris-HCl pH 7.4, 0.32M sucrose, disrupted by sonication (10 pulses, 5 times) and centrifuged (800xg, 15 minutes, 4°C); the collected supernatant was centrifuged at 105,000xg for 1h at 4°C and the pellet was re-suspended in PBS. The

fluorescent assay to measure FAAH activity was performed in 96 wells black plates: 2.5 μg of human FAAH-1 membrane preparation were pre-incubated for 50 min at 37 °C, in 180 μL of assay buffer (50mM Tris-HCl pH 7.4, 0.05% Fatty acid-free BSA) with 10 μL of inhibitor or 10 μL DMSO to measure FAAH total activity. The background (no activity) samples were prepared using 180 μL of assay buffer without human FAAH-1 and 10 μL of DMSO. The reaction was then started by addition of 10 μL of the substrate (7-amino-4-methyl coumarin-arachidonamideN. 10005098, Cayman Chemical) 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 460nm). IC_{50} values (concentrations causing half-maximal inhibition) were determined by non-linear 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 from Charles River Italia were treated intraperitoneally (i.p.) with the test compound (3 mg/kg) or vehicle (1:1:8, PEG400, Tween® 80 and Saline 0.9%). One hour after treatment, 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 1000x*g* for 10 min (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 (N.90050, Cayman Chemical) and 0.6 nM anandamide [ethanolamine-1-^{3}H] (American Radiolabeled Chemicals Inc., ART. 0626, 1 mCi/mL, specific activity 60 Ci/mmoL). The reaction was stopped by adding cold 1:1 chloroform/methanol. After 10 min centrifugation (845x*g* at 4°C) 600 μL of the aqueous phase was transferred into scintillation vials previously filled with 3 mL of scintillation fluid (ULTIMA GOLD, Cat.6013329, Perkin Elmer). Radioactivity was measured by liquid scintillation counting (Microbeta2 Lumijet, Perkin Elmer Inc.).

MGL activity assay—10 ng of the purified rat MGL was pre-incubated with appropriate drug for 10 min at 37 °C in 50 mM Tris-HCl, pH 8.0 containing 0.5 mg/mL fatty acid-free bovine serum albumin (BSA, Sigma-Aldrich). The final concentration of vehicle (1% DMSO) had no effect on MGL activity. Then, 2-oleoylglycerol (2-OG, 10 μM final) was added to the mixture and incubated for additional 30 min at 37 °C. Reactions were stopped by adding chloroform:methanol (2:1, vol:vol), containing heptadecanoic acid (5 nmol/sample) as an internal standard. After centrifugation at 2,000 x *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 chloroform:methanol (1:3, vol:vol) and analyzed by a liquid chromatography/mass spectrometry (LC/MS) method.

***In vitro* Rat Plasma stability assay—**Compounds were diluted in rat plasma added 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 time points (immediately after dilution, 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 internal standard. After vortexing for 30 s, the solution was centrifuged at 3500 g for 15 min at 4 °C and the supernatant transferred for LC-MS analysis on a Waters ACQUITY UPLC/MS TQD system consisting of a TQD (Triple Quadrupole Detector) Mass Spectrometer equipped with an Electrospray Ionization interface. Briefly, 3.0 μL of the supernatant were injected on a reversed phase column (BEH C_{18} 1.7 μm 2.1X50 mm) and separated with a linear acetonitrile gradient. Compounds were quantified on the basis of their MRM (Multiple Reaction Monitoring) 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: compound remaining (%) with corresponding standard deviation at 420 minutes is reported.

***In vitro* Mouse Liver Microsomes (MLM) stability assay—**Compounds were preincubated with microsomes in 100mM TRIS-HCl pH 7.4 for 15 minutes. At time zero, cofactors were added. The final incubation conditions for each sample were: 1.25mg/mL liver microsomes, 5μM compound (final DMSO 0.1%), NADP 1mM, G6P 20mM, $MgCl_2$ 2mM, G6P dehydrogenase 2 Units. The mixture was kept at 37°C under shaking. Aliquots (30μL) were taken at various time points (typically 0, 5, 15, 30, and 60 minutes) and crashed with 200μL of acetonitrile spiked with 200nM 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 and centrifugation, 3μL of superrnatant were analyzed by LC-MS/MS by multiple reaction monitoring (MRM).

Aqueous Kinetic Solubility assay—The kinetic solubility in Phosphate Buffered Saline (PBS) at pH 7.4 was determined starting from a 10 mM DMSO solution of the test compounds. The study was performed by incubation of an aliquot of 10 mM DMSO solution in PBS (pH 7.4) at 25°C for 24h, under shaking, followed by centrifugation and quantification of dissolved compound in the supernatant by UPLC/MS. The compound target concentration in the solutionwas 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 215nm. 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 1:1 CH_3CN-H_2O, 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 (Single Quadrupole Detector) Mass Spectrometer equipped with an Electrospray Ionization interface and a Photodiode Array Detector. The PDA range was 210-400 nm. The analyses were run on an ACQUITY UPLC BEH C_{18} column (50x2.1 mmID, particle size 1.7 μm) with a VanGuard BEH C_{18} pre-column (5x2.1 mmID, 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). Electrospray ionization in positive and negative mode was applied in the mass scan range 100-500Da.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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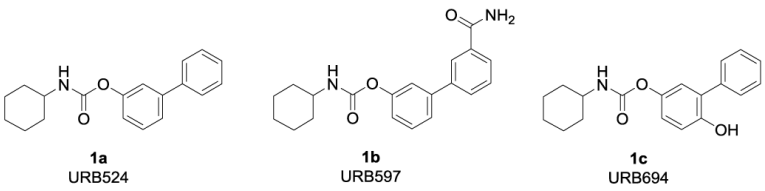


1a URB524 **1b** URB597 **1c** URB694

Figure 1.
O-arylcarbamates FAAH inhibitors.

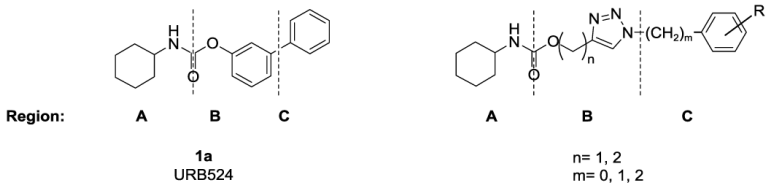
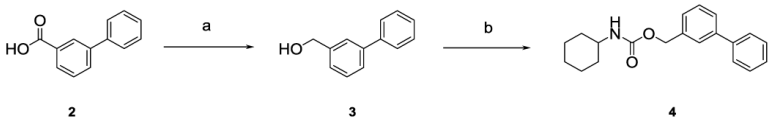
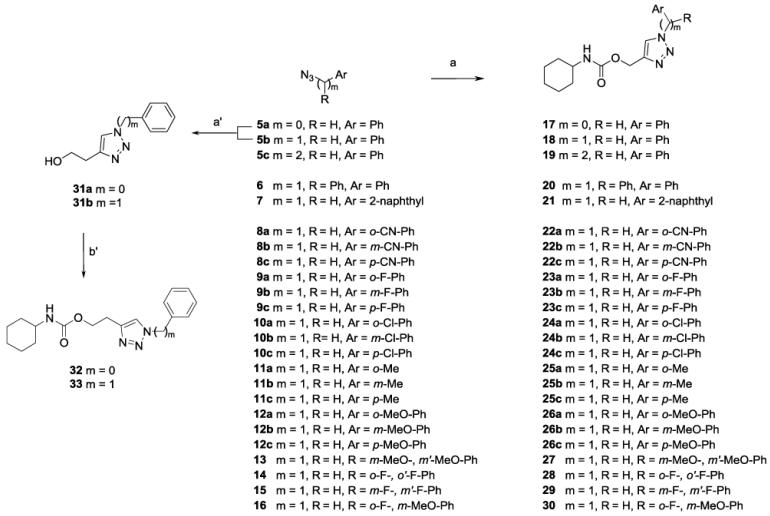


Figure 2.
Structures of URB524 (**1a**) and *O*-(1,2,3-triazol-4-yl)alkyl carbamate derivatives



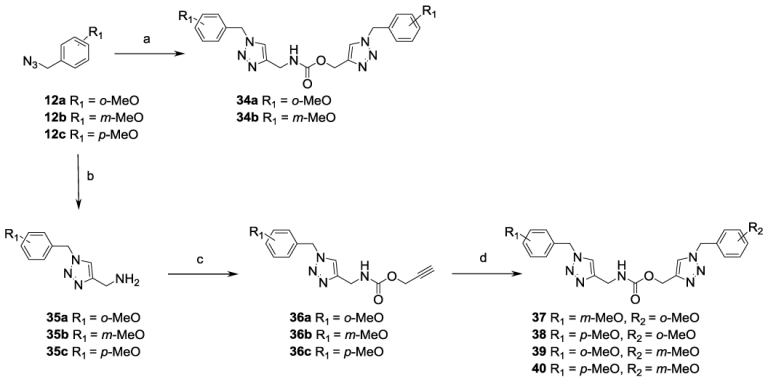
Scheme 1.

Synthesis of (3-phenylphenyl)methyl *N*-cyclohexylcarbamate (**4**). *Reagents and conditions*: a) $LiAlH_4$ (2N solution in THF), dry THF, 0 °C to rt, 2h; (b) cyclohexyl isocyanate, DMAP, dry CH3CN, 80 °C, 6h.



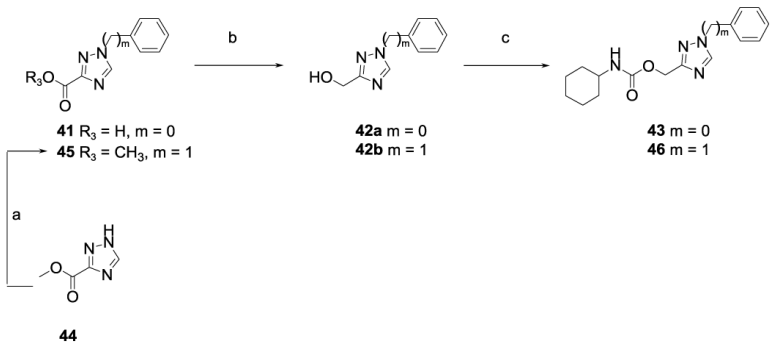
Scheme 2.

Synthesis of *O*-(triazol-4-yl)alkyl carbamates **17**-**30**, **32**-**33**. *Reagents and conditions*: a) prop-2-yn-1-yl *N*-cyclohexylcarbamate, sodium L-ascorbate, copper (II) sulfate pentahydrate, H_2O-*tert*-BuOH 1:1, rt, 3h; a') but-3-yn-1-ol, sodium L-ascorbate, copper (II) sulfate pentahydrate, H_2O-*tert*-BuOH 1:1, rt, 3h; b') cyclohexyl isocyanate, DMAP, dry MeCN, 80 °C, 5h.



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, copper (II) sulfate pentahydrate, H_2O-*tert*-BuOH 1:1, rt, 3h; b) prop-2-yn-1-amine, sodium L-ascorbate, copper (II) sulfate pentahydrate, H_2O-*tert*-BuOH 1:1, rt, 3h; c) prop-2-ynyl chloroformate, Et_3N, dry DCM, 0 °C, 30 min; d) **12a** or **12b**, sodium L-ascorbate, copper (II) sulfate pentahydrate, H_2O-*tert*-BuOH 1:1, rt, 3h.

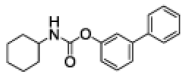
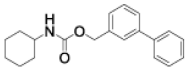
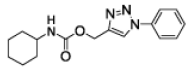
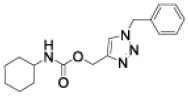
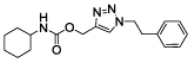
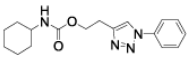
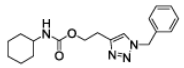
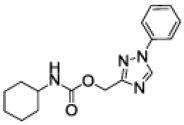
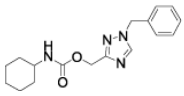


Scheme 4.

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, 16h; b) $LiAlH_4$ (2N solution in THF), dry THF, 0 °C to rt, 2h; c) cyclohexyl isocyanate, DMAP, dry CH_3CN, 80 °C, 6h.

Table 1

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

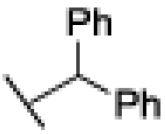
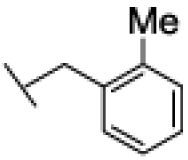
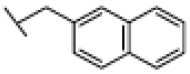
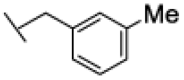
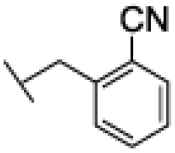
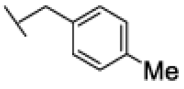
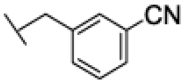
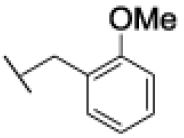
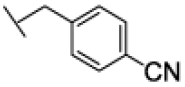
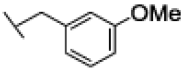
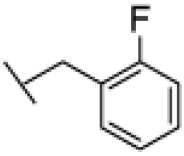
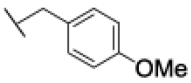
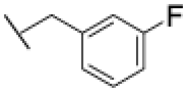
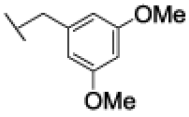
Compounds	Structure	IC_{50} [nM][a]	Plasma half-life (min.)	% remaining after 7 h[b]
1a		5.7 ± 1.2	62 ± 19	0
4		65% @ 100 μM	-	-
17		380.6 ± 62.9	-	90 ± 9
18		26.2 ± 3.8	-	92 ± 8
19		833 ± 129	-	-
32		1278 ± 84	-	-
33		2535 ± 106	-	-
43		70% @ 100 μM	-	-
46		51500 ± 1125	-	-

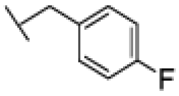
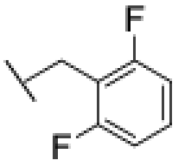
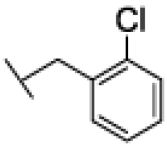
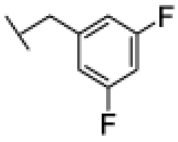
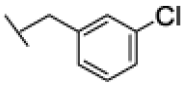
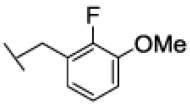
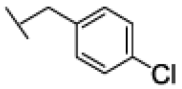
[a] IC_{50} values are reported as the mean ± SEM (n= 3);

[b] Percent of parent compound remaining after 7 h incubation.

Table 2

FAAH inhibitory activity of *O*-(triazol-4-yl)methyl carbamates variously substituted at region C.

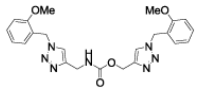
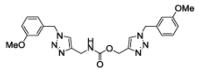
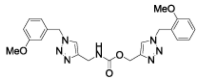
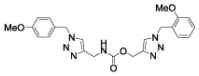
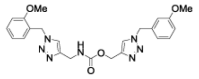
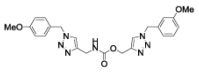
Cpds	R	IC_{50} [nM][a]	Cpds	R	IC_{50} [nM][a]
20		73% @ 100 μM	**25a**		21.5 ± 1.2
21		2003 ± 636	**25b**		24.5 ± 6.2
22a		19.5 ± 4.3	**25c**		500 ± 44
22b		21.5 ± 7.9	**26a**		1.4 ± 0.2
22c		2282 ± 585	**26b**		11.8 ± 3.7
23a		9.9 ± 1.9	**26c**		627 ± 173
23b		12.1 ± 0.8	**27**		10.5 ± 2.9

Cpds	R	IC_{50} [nM][a]	Cpds	R	IC_{50} [nM][a]
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 reported as the mean ± SEM (n= 3).

Table 3

FAAH inhibitory activity of *N*-(triazol-4-yl)methyl *O*-(triazol-4-yl)methyl carbamates **34a-40**.

Compounds	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 reported as the mean ± SEM (n= 3).

Table 4

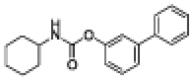
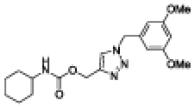
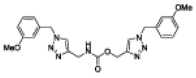
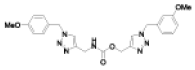
Comparison of *r*-FAAH vs. *h*-FAAH-1 inhibitory activity of selected compounds.

Compounds	*r*-FAAH IC_{50} [nM][a]	*h*-FAAH IC_{50} [nM][a]
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 reported as the mean ± SEM (n= 3).

Table 5

Characterization of selected compounds

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

[a] IC_{50} values for FAAH inhibition are reported as the mean ± SEM (n= 3);

[b] Data are the mean ± SEM of three determinations;

[c] Data are the mean of three determinations;

[d] Phosphate buffered saline, pH 7.4, single determination.