European Journal of Medicinal Chemistry 111 (2016) 138–159

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Research paper

Potent α-amino-β-lactam carbamic acid ester as NAAA inhibitors. Synthesis and structure–activity relationship (SAR) studies

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ARTICLE INFO

Article history:
Received 23 November 2015
Received in revised form
18 January 2016
Accepted 24 January 2016
Available online 27 January 2016

Keywords:
N-acylethanolamine acid amidase
Cysteine hydrolase
Inhibitors
β-lactams
Carbamates
Structure–activity relationships

ABSTRACT

4-Cyclohexylbutyl-*N*-[(*S*)-2-oxoazetidin-3-yl]carbamate (**3b**) is a potent, selective and systemically active inhibitor of intracellular NAAA activity, which produces profound anti-inflammatory effects in animal models. In the present work, we describe structure–activity relationship (SAR) studies on 3-aminoazetidin-2-one derivatives, which have led to the identification of **3b**, and expand these studies to elucidate the principal structural and stereochemical features needed to achieve effective NAAA inhibition. Investigations on the influence of the substitution at the β-position of the 2-oxo-3-azetidinyl ring as well as on the effect of size and shape of the carbamic acid ester side chain led to the discovery of **3ak**, a novel inhibitor of human NAAA that shows an improved physicochemical and drug-like profile relative to **3b**. This favourable profile, along with the structural diversity of the carbamic acid chain of **3b**, identify this compound as a promising new tool to investigate the potential of NAAA inhibitors as therapeutic agents for the treatment of pain and inflammation.

1. Introduction

Identifying novel molecular targets for the treatment of pain and inflammation is of pivotal therapeutic importance and remains a major challenge for researchers in industry and academia [1]. Since its discovery in 2005 [2], the intracellular cysteine amidase *N*-acylethanolamine acid amidase (NAAA) has attracted increasing attention as a potential anti-inflammatory target, due to its essential role in the regulation of lipid-amide signalling at peroxisome proliferator-activated receptor-α (PPAR-α) in macrophages and other host-defence cells [2–8].

NAAA belongs to the N-terminal nucleophile (Ntn) family of enzymes and catalyses the deactivating cleavage of saturated and monounsaturated fatty acid ethanolamides (FAEs) into the corresponding free fatty acids and ethanolamine [3,4,9,10], thus interrupting their peroxisome proliferator-activated receptor–α (PPAR-α) mediated actions. NAAA is primarily localized to macrophages [11] and B-lymphocytes [8] and, like other Ntn enzymes, is activated by auto-proteolysis, which occurs at acidic pH and generates a catalytically competent form of the enzyme [12].

Abbreviations Used: NAAA, *N*-acylethanolamine acid amidase; FAEs, fatty acid ethanolamides; OEA, oleoylethanolamide; PEA, palmitoylethanolamide; FAAH, fatty acid amide hydrolase; AC, acid ceramidase; PPAR–α, peroxisome proliferator-activated receptor–α; PAMCA, *N*-(4-methyl-2-oxo-chromen-7-yl)-hexadecanamide; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; 2-DPC, di-2-pyridyl carbonate.

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http://dx.doi.org/10.1016/j.ejmech.2016.01.046
0223-5234/

The FAEs are a class of bioactive lipids [13–15]that contribute to the control of numerous physiological functions, including pain and inflammation [16–19]. Among these lipids, NAAA preferentially degrades palmitoylethanolamide (PEA) and oleoylethanolamide (OEA) [2–4]. PEA is produced by many mammalian cells [20], and has been shown to inhibit peripheral inflammation and mast cell degranulation [21,22] and to exhibit antinociceptive properties in rat and mouse models of acute and chronic pain [23–25]. In addition, evidence suggests that PEA may attenuate skin inflammation and neuropathic pain in humans [26–28]. These effects are mainly dependent on the ability of PEA to activate PPAR–α [19,29–31]. Recent studies have shown that sustaining endogenous PEA levels in vivo by protection from NAAA-catalysed degradation attenuates lung inflammation [8] and hyperalgesic and allodynic states elicited in mice and rats by local inflammation or nerve damage [6,32].

Despite the encouraging pharmacological benefits achieved by restoring intracellular FAE levels by inhibition of NAAA, only few chemical classes of potent inhibitors have been discovered [33]. Among them, compounds featuring an electrophilic warhead, such as α-amino-β-lactone derivatives, displayed high potency as NAAA inhibitors [5,34–37]. Topical administration of the potent and selective β-lactone NAAA inhibitor, 5-phenylpentyl-*N*-[(2*S*,3*R*)-2-methyl-4-oxo-oxetan-3-yl]carbamate (ARN077, **1**, Fig. 1) [35,38,39], was reported to elevate PEA and OEA levels in mouse skin and sciatic nerve tissues, and to attenuate nociception in mice and rats through a mechanism that required PPAR–α activation [6].

However, α-amino-β-lactone derivatives have limited chemical and plasma stability [36,40,41], due to the hydrolytic cleavage of the β-lactone ring, which restricts their in vivo use to topical applications [6,35]. To overcome the limited stability of this class of inhibitors, we investigated compounds in which an α-amino-β-lactam (3-aminoazetidin-2-one) group replaces the β-lactone ring. These compounds retain key structural features of the β-lactone series, and showed good human-NAAA (*h*-NAAA) inhibitory activity and favourable physicochemical properties for systemic administration [42]. In particular, *N*-[(*S*)-2-oxoazetidin-3-yl]nonanamide (**2**, Fig. 1) displayed an acceptable inhibitory potency ($IC_{50} = 0.34$ μM) against *h*-NAAA, good stability in buffer (PBS at pH 7.4 and 5.0) and in mouse and rat plasma, and good oral bioavailability after single oral administration in rats [42].

Interestingly, the replacement of the exocyclic amide functionality with a carbamic acid ester, as in derivative **3a** (Fig. 1) was beneficial for NAAA inhibitory activity ($IC_{50} = 0.122$ μM) [42,43].

This prompted us to investigate further the structural and stereochemical features of this new series of derivatives, leading after a modification on the carbamic acid side chain of **3a** to the discovery of 4-cyclohexylbutyl-*N*-[(*S*)-2-oxoazetidin-3-yl]carbamate (ARN726, **3b**, Fig. 1), as a novel and potent NAAA inhibitor [8]. Compound **3b** inhibited NAAA activity both in vitro and in vivo as demonstrated by a competitive activity-based protein profiling (ABPP) study with a structurally similar chemical probe [44]. Consistent with those findings, **3b** exerted marked anti-inflammatory effects in mouse models of inflammation and suppressed LPS-stimulated inflammatory reactions in human macrophages [8].

In the present work, we describe the structure–activity relationship (SAR) work that has led to the identification of β-lactam **3b** and the elucidation of key structural and stereochemical features needed to achieve *h*-NAAA inhibition with this class of compounds. In particular, we investigated the influence of the substitution at β-position of the 2-oxo-3-azetidinyl ring as well as the effect of the size and shape of the carbamic acid ester side chain. We also outline the selectivity, stability, and solubility of the best among these novel NAAA inhibitors, which might aid further experimental investigations of the roles of NAAA.

2. Chemistry

The synthesis of differently substituted β-lactam carbamic acid esters was efficiently accomplished in an enantio- and diastereoselective fashion by a general strategy consisting of coupling an appropriate 3-amino-β-lactam core with a desired activated alcohol. Initially, a series of β-lactam carbamates bearing a 4-cyclohexylbutyl side chain (**3b**–**10b**) was synthesized as reported in Scheme 1.

The commercially available 4-cyclohexylbutan-1-ol (**11b**) was reacted with di-2-pyridyl carbonate (2-DPC) and catalytic DMAP to give an isomeric mixture (1.7:1 ratio) of 4-cyclohexylbutyl 2-pyridyl carbonate (**12b**) and 4-cyclohexylbutyl 2-oxopyridine-1-carboxylate (**13b**) [38,42,45]. Then, the coupling reaction with (*S*)- or (*R*)-2-oxoazetidin-3-yl ammonium acetate (**14** and **15**) afforded enantiomeric 4-cyclohexylbutyl-*N*-[(S/R)-2-oxoazetidin-3-yl]carbamates (**3b** and **4b**, respectively) (Scheme 1) [8,42]. The reaction of the isomeric mixture **12b** and **13b** with (2*S*,3*S*)-(2-methyl-4-oxo-azetidin-3-yl) ammonium tosylate (**16**), its enantiomer (2*R*,3*R*)-**17**, and the corresponding epimers (2*R*,3*S*)-**18** and (2*S*,3*R*)-**19** [46] afforded the desired β-methyl β-lactam carbamates **5b**–**8b** in moderate to good yields (Scheme 1). Finally, the coupling reaction with both *N*-protected 2,2-dimethyl (*S*)-β-lactam amine **20** and its (*R*)-enantiomer **21** yielded, after oxidative cleavage of the *bis*(-trimethylsilyl)methyl group, the 2,2-dimethyl β-lactam carbamates **9b** and **10b** (Scheme 1) [47].

(2*S*,3*S*)-2-Methyl-4-oxo-azetidin-3-yl ammonium tosylate **16** was prepared following a five-step sequence [48,49], starting from the commercially available *N*-Cbz-L-threonine, which was initially converted into the corresponding *O*-methyl hydroxamate derivative (**26**) in good yield (Scheme 2).

Mesylation of **26** gave compound **30**, which was cyclized under basic conditions (K_2CO_3) to afford the desired 2-azetidinone (**34**) in high yields (73%, over 2 steps). Selective *N*-methoxy deprotection was carried out using a fresh solution of samarium iodide, leading to the formation of the β-methyl β-lactam **38** [49]. Cbz-deprotection of **38** and *in situ* trapping of the resulting 2-methyl-3-aminoazetidin-4-one with *p*-toluensulfonic acid gave the tosylate salt **16** in quantitative yield (Scheme 2).

Tosylate salts **17**–**19** were stereoselectively obtained using the same procedure, starting either from the commercially available *N*-

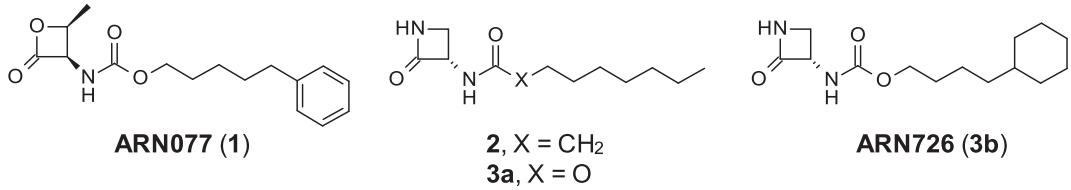
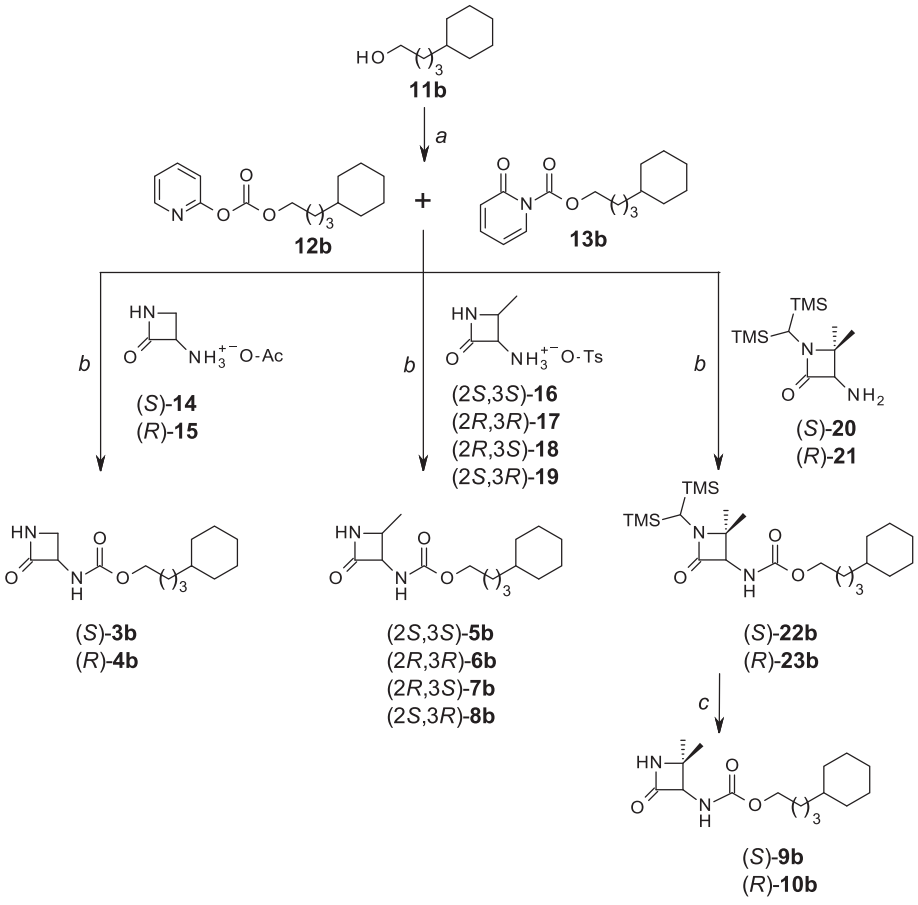
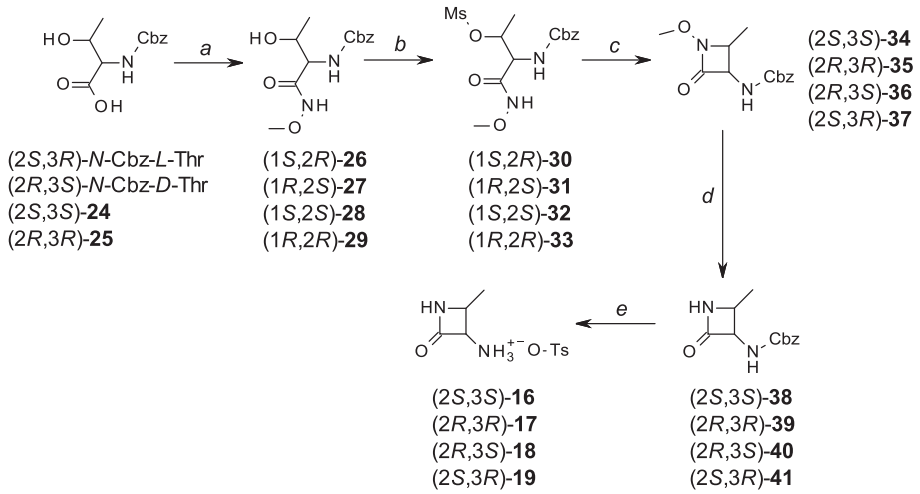


Fig. 1. Potent β-lactone- (1) and β-lactam-based (**2**, **3a** and **3b**) NAAA inhibitors.



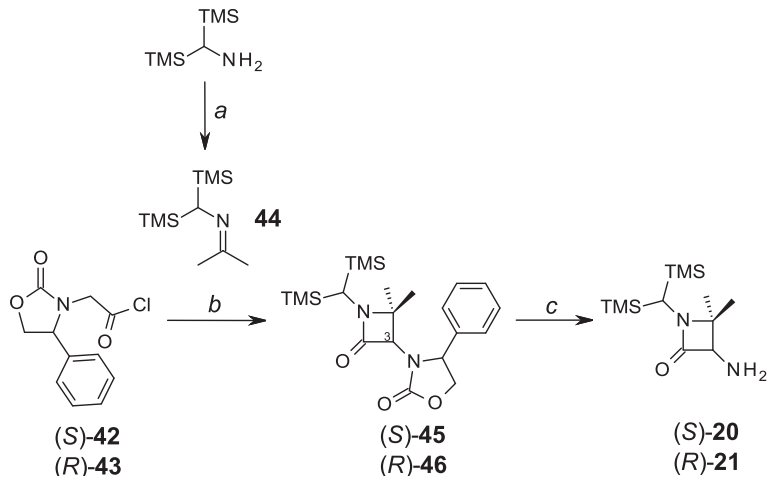
Scheme 1. General Synthetic Pathways to β-Lactam Carbamic Acid Esters **3b**–**10b**. Reagents and conditions: (a) 2-DPC, DMAP, dry CH_2Cl_2, room temperature, 16 h; (b) DIPEA, dry CH_2Cl_2, room temperature, 16 h; (c) CAN, CH_3CN/H_2O (4:1), 0 °C to room temperature, 1 h, then acetone/sat. sol. $NaHCO_3$ (1:1), room temperature, 16 h [TMS = Trimethylsilyl].



Scheme 2. Synthesis of Isomeric 2-Methyl-4-oxo-azetidin-3-yl Ammonium Tosylate Salts **16**–**19**. Reagents and conditions: (a) CH_3ONH_2HCl, EDC, THF/H_2O (3:1), room temperature, 3 h; (b) $CH_3S(O)_2Cl$, dry Pyridine, -5 °C–0 °C, 3 h; (c) K_2CO_3, Acetone, 100 °C, 3 h; (d) SmI_2 (0.5 M in THF), dry THF, room temperature, 30 min; (e) 1,4-Cyclohexadiene, 10% Pd on charcoal, EtOH, room temperature, 2 h, then *p*-TsOH, EtOAc, room temperature, 1 h [Cbz = Benzyloxycarbonyl; Ms = Mesyl; Ts = *p*-Tosyl].

Cbz-D-threonine or the synthesized L- and D-*N*-Cbz-*allo*-threonine (**24** and **25**), respectively (Scheme 2) (see Supporting Information).

The (*S*)-3-amino-1-(bis(trimethylsilyl)methyl)-4,4-dimethylazetidin-2-one **20** was prepared by modifications of previously reported procedures. The synthetic sequence was based on a [2+2]-cycloaddition reaction between the properly protected imine **44** and the Evans-Sjögren ketene, generated *in situ* from the enantiomerically pure oxazolidinone **42** (Scheme 3) [47,50]. Final removal of Evans chiral auxiliary under modified Birch reduction conditions (Na-SG, *t*-AmOH, EDA, see Supporting Information),



Scheme 3. Synthesis of (*S*)- and (*R*)-3-Amino-1-[bis(Trimethylsilyl)methyl]-4,4-dimethyl-azetidin-2-one (**20**,**21**). Reagents and conditions: (a) Acetone, room temperature, 12 h; (b) dry Et_3N, 4 Å MS, dry CH_2Cl_2, 0 °C–55 °C, 12 h; (c) Na-SG(I) 35–40%, ethylendiamine, dry THF, 0 °C, 15 min, then 2-methyl-2-butanol, 0 °C, 5 min, then **45** or **46** in dry THF, 0 °C to room temperature, 2 h [TMS = Trimethylsilyl].

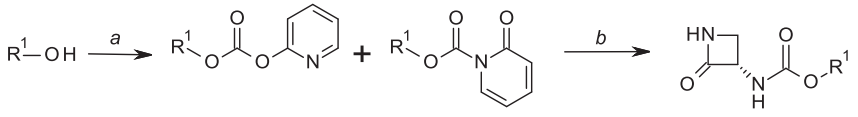
afforded the (*S*)–N-protected 3-amino-β-lactam **20** [51]. The enantiomeric (*R*)-β-2,2-dimethyl β-lactam amine **21** was obtained following the same synthetic pathway starting from intermediate (*R*)-**43** [52].

A series of differently substituted (*S*)-aminoazetidin-2-one carbamate derivatives (**3b**–**am**) was easily accessed as shown in Scheme 4. Various alcohols (**11b**–**am**) were allowed to react with 2-DPC to give a crude mixture of pyridyl carbonates **12b**–**am** and 2-oxopyridine-1-carboxylates **13b**–**am**, which was subsequently coupled with the acetate salt (*S*)-**14** (Scheme 4).

Most of the used alcohols were commercially available (**11b**–**f**,**l**,**n**,**p**,**r**,**y**,**z**,**ab**,**ac**,**ae**,**aj**) or prepared by reduction of the corresponding commercially available precursors (**11g**,**s**,**v**,**w**,**aa**,**ad**,**af**,**ak**,**al**, see Supporting Information). The remaining alcohols were either obtained following previously reported protocols (**11i** [53], **j** [54], **t** [55], **u** [56], **ah** [37], see Supporting Information) or synthesized as described in Schemes 5 and 6 (**11h**,**k**,**m**,**o**,**q**,**x**,**ag**,**ai**,**am**).

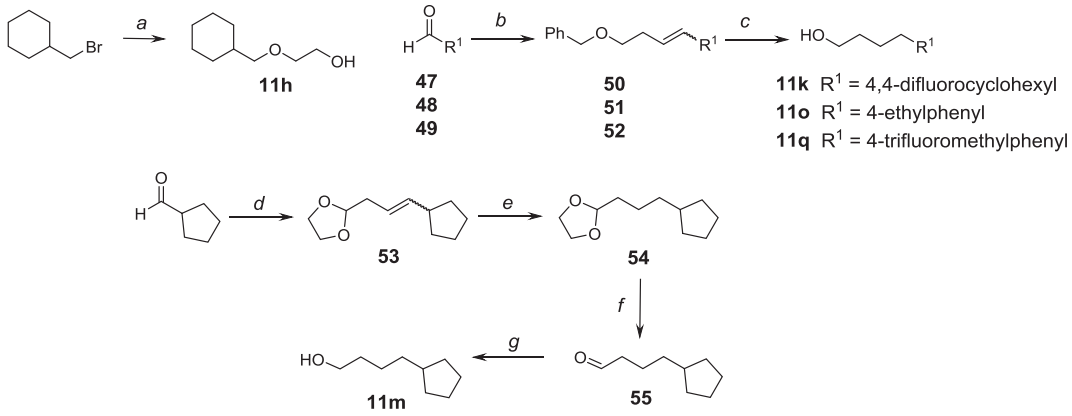
Compound **11h** was prepared in a straightforward manner by heating bromo-methylcyclohexane in ethylene glycol, under strong basic conditions (Scheme 5) [57,58].

Alcohols **11k**,**o**,**q** were obtained in moderate to good yields starting from the corresponding aldehydes (**47**–**49**) *via* Wittig olefination with a benzyl-protected phosphonium salt, followed by a one-pot reduction/deprotection reaction, using $Pd(OH)_2$ in the H-Cube flow reactor (Scheme 5). A similar synthetic strategy was used for compound **11m**, where cyclopentanecarbaldehyde was reacted with 2-(1,3-dioxolan-2-yl)-ethyl-(triphenyl)-phosphonium bromide to give olefin **53** (7:93 *E*/*Z* ratio). Reduction of the derivative **53**, followed by dioxolane cleavage, afforded aldehyde **55** which upon reduction with $NaBH_4$ furnished the desired compound (Scheme 5).

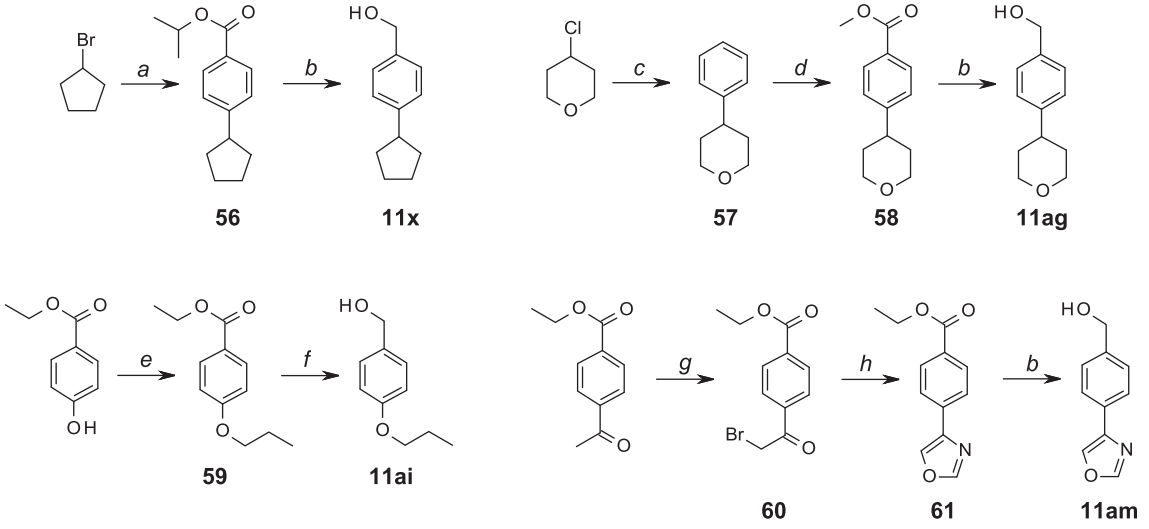


b: R^1 = 4-cyclohexylbutyl
c: R^1 = *n*-octyl
d: R^1 = 5-phenylpentyl
e: R^1 = (4-phenylphenyl)methyl
f: R^1 = 3-cyclohexylpropyl
g: R^1 = 5-cyclohexylpentyl
h: R^1 = 2-(cyclohexylmethoxy)ethyl
i: R^1 = 3-(cyclohexoxy)propyl
j: R^1 = 4-tetrahydropyran-4-yl-butyl
k: R^1 = 4-(4,4-difluorocyclohexyl)butyl
l: R^1 = 4-butylcyclohexyl
m: R^1 = 4-cyclopentylbutyl
n: R^1 = 4-phenylbutyl
o: R^1 = 4-(4-ethylphenyl)butyl
p: R^1 = 4-(4-methoxyphenyl)butyl
q: R^1 = 4-(4-trifluoromethylphenyl)butyl
r: R^1 = 4-(2-pyridyl)butyl
s: R^1 = (*E*)-4-phenylbut-3-enyl
t: R^1 = (*Z*)-4-phenylbut-3-enyl
u: R^1 = (3-cyclohexylphenyl)methyl
v: R^1 = (2-cyclohexylphenyl)methyl
w: R^1 = (4-cyclohexylphenyl)methyl
x: R^1 = (4-cyclopentylphenyl)methyl
y: R^1 = (4-methylphenyl)methyl
z: R^1 = (4-ethylphenyl)methyl
aa: R^1 = (4-propylphenyl)methyl
ab: R^1 = (4-*n*-butylphenyl)methyl
ac: R^1 = (4-*tert*-butylphenyl)methyl
ad: R^1 = tetralin-6-yl-methyl
ae: R^1 = indan-5-yl-methyl
af: R^1 = 1-(4-cyclohexylphenyl)ethyl
ag: R^1 = (4-tetrahydropyran-4-yl-phenyl)methyl
ah: R^1 = (4-cyclohexoxyphenyl)methyl
ai: R^1 = (4-propoxyphenyl)methyl
aj: R^1 = 1,3-benzodioxol-5-yl-methyl
ak: R^1 = (2,2-difluoro-1,3-benzodioxol-5-yl)methyl
al: R^1 = [4-(3-pyridyl)phenyl]methyl
am: R^1 = (4-oxazol-4-ylphenyl)methyl

Scheme 4. Synthesis of (*S*)-2-Oxoazetidin-3-yl-carbamic Acid Esters **3b**–**am**. Reagents and conditions: (a) 2-DPC, DMAP, dry CH_2Cl_2, room temperature, 16 h; (b) (*S*)-**14**, DIPEA, dry CH_2Cl_2, room temperature, 16 h.



Scheme 5. Synthesis of Alcohols **11 h,k,m,o,q**. Reagents and conditions: (a) ethylene glycol, 25.5 M NaOH, 80 °C, 16 h; (b) [3-benzyloxypropyl-$(Ph)_3$P]Br, *n*-BuLi (2.5 M in hexane), dry THF, -30 °C, 45 min, then **47**–**49**, -30 °C, 45 min, then room temperature, 16 h; (c) H_2, 10% $Pd(OH)_2$/C (H-Cube, EtOH, 60 °C, 1.0 bar); (d) [2-(1,3)-dioxolan-2-yl)-ethyl-$(Ph)_3$P]Br, *n*-BuLi (2.5 M in hexane), dry THF, -30 °C, 45 min, then cyclopentanecarbaldehyde, -30 °C, 45 min, then room temperature, 16 h; (e) H_2, 10% Pd/C (H-Cube, EtOH, 35 °C, 1.0 bar); (f) 70% AcOH, THF, 65 °C, 16 h; (g) $NaBH_4$, EtOH, room temperature, 30 min.



Scheme 6. Synthesis of Alcohols **11x,ag,ai,am**. Reagents and conditions: (a) 4-methoxycarbonyl-phenyl boronic acid, sodium hexamethyldisylazane, *trans*-2-hydroxy-cyclohexylamine HCl, NiI_2, *i*-PrOH, room temperature, 5 min, then bromocyclopentane, 60 °C, 6 h; (b) $LiAlH_4$, dry THF, 0 °C, then room temperature, 3 h; (c) phenyl boronic acid, KHMDS, (*S*)-(+)-prolinol, $NiCl_2$-glyme, *i*-PrOH, room temperature, 10 min, then 4-chlorotetrahydropyran, 65 °C, 50 h; (d) $(COCl)_2$, dry CH_2Cl_2, -20 °C, 30 min, then $AlCl_3$, -20 °C, 1 h, then room temperature, 1 h, then MeOH, -20 °C, 16 h; (e) 1-bromopropane, NaH, room temperature, 18 h, then 80 °C, 3 h; (f) $LiAlH_4$, dry Et_2O, 0 °C, then room temperature, 4 h; (g) $CuBr_2$, EtOAc, reflux, 16 h; (h) $NH_4^+HCOO^-$, HCOOH, reflux, 7 h.

The synthesis of alcohols **11x,ag** was accomplished using minor modifications of a protocol reported by Fu and co-workers (Scheme 6) [59].

Suzuki coupling between bromocyclopentane and 4-methoxycarbonyl-phenyl boronic acid, using NiI_2 as a catalyst, furnished isopropyl ester **56**, which was directly reduced with $LiAlH_4$ to give alcohol **11x**. 4-Phenyl-tetrahydropyranyl derivative **57**, obtained *via* Suzuki reaction between phenyl boronic acid and 4-chlorotetrahydropyran, was regioselectively acylated *via* a Friedel–Crafts reaction [60] and then subjected to $LiAlH_4$ reduction to give compound **11ag**. *O*-Alkylation of ethyl 4-hydroxybenzoate with 1-bromopropane led to derivative **59**, which upon reduction of the ethyl ester under standard conditions gave the alcohol **11ai**. Finally, alcohol **11am** was obtained in a reasonable yield (27%) after a three steps sequence, which consisted in the formation of the oxazole ring starting from ethyl 4-acetylbenzoate followed by $LiAlH_4$ reduction of the ethyl ester (Scheme 6) [61,62].

3. Results and discussion

In a previous study on NAAA inhibitors we reported that α-amino-β-lactam (3-aminoazetidin-2-one) amide derivatives display a set of pharmacological and physicochemical properties that render them suitable for systemic administration, overcoming the limited hydrolytic and plasma stability of α-amino-β-lactone-based NAAA inhibitors, such as ARN077 (**1**, Fig. 1) [42].

The compound *N*-[(*S*)-2-oxoazetidin-3-yl]nonanamide (**2**, Fig. 1) showed acceptable inhibitory potency ($IC_{50} = 0.34$ μM) for *h*-NAAA, good stability in buffer (at pH 7.4 and 5.0) and in mouse and rat plasma, and good oral bioavailability after single oral administration in rats. The replacement of the exocyclic nonanamide residue with a heptyl carbamate moiety, as in derivative **3a**, produced an increase in NAAA inhibitory potency ($IC_{50} = 0.122$ μM, Table 1) [43].

This result prompted us to expand this new series of derivatives in the attempt to further improve potency. We first replaced the heptyl group of the carbamic acid ester with residues that in the α-

amino-β-lactone analogue series yielded potent NAAA inhibitors [38]. The introduction of a linear aliphatic octyl moiety afforded the double-digit nanomolar inhibitor **3c** (IC_{50} = 0.037 μM), while the 5-phenylpentyl residue, which is found in compound **1**, and the *p*-biphenylmethyl group resulted in compounds **3d** (IC_{50} = 0.298 μM) and **3e** (IC_{50} = 0.51 μM), respectively (Table 1).

While the increase in potency observed with compound **3c** is in accordance with the SAR observed in α-amino-β-lactone carbamate derivatives, where long alkyl chains are preferred over short ones [38], the detrimental effect of a 5-phenylpentyl or a *p*-biphenylmethyl group differentiates the SAR of β-lactam carbamate derivatives from that of the corresponding β-lactone series. We then explored the replacement of the long unsubstituted alkyl chain with a short, ω-cycloalkyl-substituted chain, a moiety that led to potent NAAA inhibitors in the α-amino-β-lactone series [37,38]. The introduction of a 4-cyclohexylbutyl group yielded compound **3b**, which allowed recovering double-digit nanomolar potency, while reducing the length of the alkyl chain. This compound was preferred over **3c** for conducting further studies because available data on α-amino-β-lactone and α-amino-β-lactam derivatives showed that molecules bearing a long alkyl chain have lower selectivity for NAAA versus acid ceramidase, a cysteine amidase showing 33–34% identity and 70% similarity to NAAA and catalyses the hydrolysis of ceramide, a pro-inflammatory lipid messenger [63,64]. Compound **3b** was shown to block NAAA covalently *via* *S*-acylation of the catalytic cysteine residue in in vitro experiments, to suppress lung inflammation in mice *via* a mechanism that requires PEA- and OEA-mediated PPAR–α activation, and to prevent endotoxin-induced inflammatory responses in human macrophages [8].

Encouraged by these results, we undertook a SAR study in order to elucidate the stereochemical and structural requirements necessary for NAAA inhibition by compound **3b** and a series of analogues. We explored both the influence of the β-substitution on the β-lactam ring and the effect of modifications on the carbamic side chain, taking into account structural changes such as the introduction of heteroatoms, the length of the side-chain, and the insertion of conformationally constrained moieties.

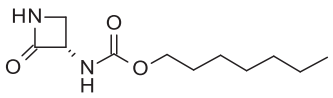
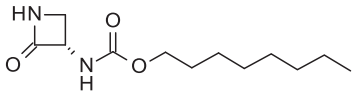
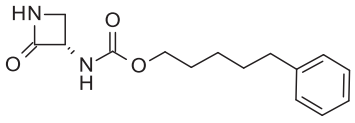
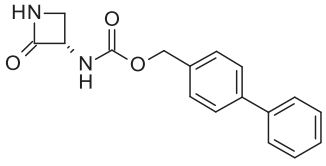
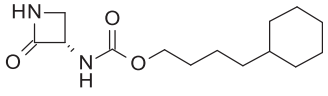
All synthesized compounds were tested for their ability to inhibit the hydrolysis of *N*-(4-methyl-2-oxo-chromen-7-yl)-hexadecanamide (PAMCA) by recombinant human NAAA heterologously expressed in HEK293 cells (*h*-NAAA) (see Supporting Information). Median inhibitory concentration (IC_{50}) values are reported in Tables 1–5.

A small set of representative compounds was also evaluated for selectivity, and physicochemical and drug-like properties, such as solubility in buffer, and mouse and rat plasma and liver microsomal stability (Table 5).

As a first step in our study, we modified the α-amino β-lactam scaffold, while maintaining fixed the 4-cyclohexylbutyl carbamate side chain. We explored the role of the stereochemistry at α-position and the effect of mono/di-substitution at β-position of the azetidinone ring.

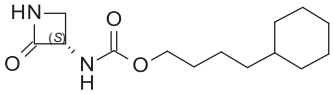
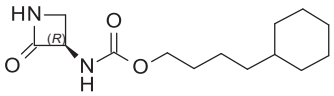
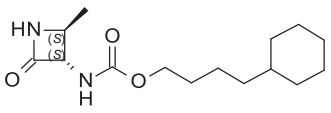
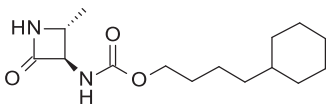
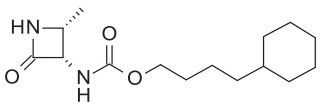
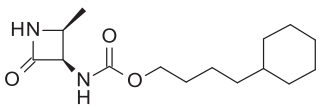
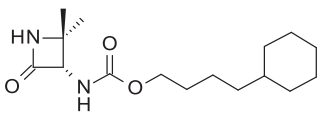
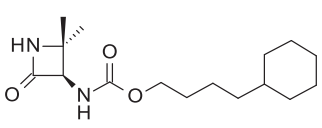
A strong stereo-recognition at the NAAA active-site was clearly proved with compound **4b**, the (*R*)-enantiomer of **3b**, which showed a 40-fold drop in activity (IC_{50} = 3.11 μM, Table 2) compared to its stereoisomer, in agreement with our previous observation with the 3-aminoazetidin-2-one amide derivative **2** and its enantiomer [42]. The importance of the (*S*)-configuration at α-position for inhibition of NAAA was also confirmed in vivo, as **4b** showed no significant anti-inflammatory effect in mouse models in

Table 1
Inhibitory potencies (IC_{50}) of compounds **3a,e** on *h*-NAAA activity.[a]

Compounds	Structure	IC_{50} (μM) ± SD
3a		0.122 ± 0.022
3c		0.037 ± 0.006
3d		0.298 ± 0.037
3e		0.513 ± 0.108
3b		0.073 ± 0.008

[a] IC_{50} values are reported as mean values of three or more determinations.

Table 2
Inhibitory potencies (IC_{50}) of compounds **3b**–**10b** on *h*-NAAA activity.[a]

Compounds	Structure	IC_{50} (μM) ± SD
3b		0.073 ± 0.008
4b		3.11 ± 0.94
5b		0.24 ± 0.069
6b		29.36 ± 2.74
7b		>50
8b		12.91 ± 2.79
9b		25.42 ± 1.02
10b		17.30 ± 2.23

[a] IC_{50} values are reported as mean values of three or more determinations.

Table 3
Inhibitory potencies (IC_{50}) of compounds **3f**–**t** on *h*-NAAA activity.[a]

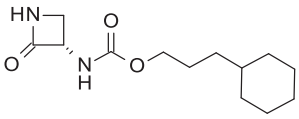
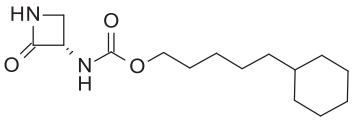
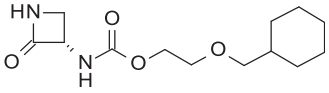
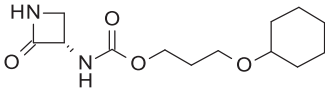
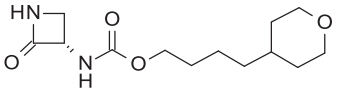
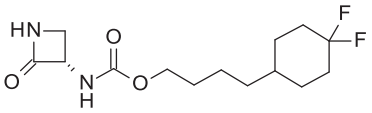
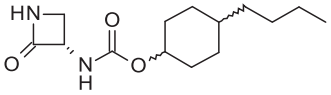
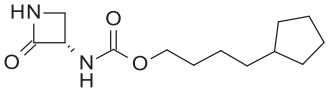
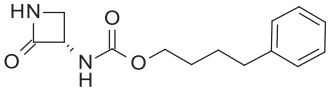
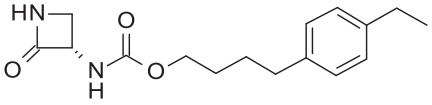
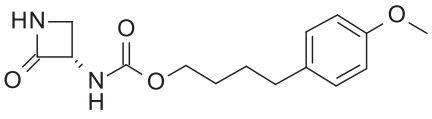
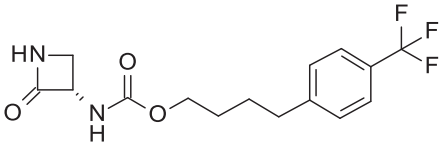
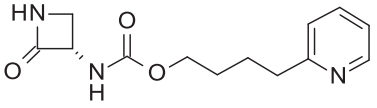
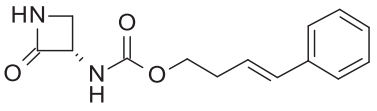
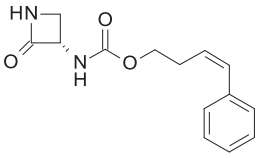
Compounds	Structure	IC_{50} (μM) ± SD
3f		0.044 ± 0.005
3g		0.049 ± 0.008
3h		1.28 ± 0.122
3i		2.67 ± 0.343
3j		9.51 ± 1.23
3k		0.33 ± 0.077
3l		0.323 ± 0.045
3m		0.033 ± 0.005
3n		1.11 ± 0.342
3o		0.23 ± 0.023
3p		0.52 ± 0.065
3q		0.073 ± 0.003
3r		30.65 ± 9.56
3s		2.89 ± 0.128

Table 3 (*continued*)

Compounds	Structure	IC_{50} (μM) ± SD
3t		1.51 ± 0.17

[a] IC_{50} values are reported as mean values of three or more determinations.

contrast to its enantiomer **3b** [8].

Then, as α-amino β-lactones bearing a methyl group in β-position of the lactone ring showed increased NAAA inhibition compared to the corresponding unsubstituted derivatives [35,38], we turned our attention to such modification on the β-lactam core. Accordingly, we synthesized a small series of 2-methyl- and 2,2-dimethyl-β-lactam carbamic acid esters (Table 2). The insertion of a single methyl group at C(3) position was in general not beneficial for potency. Among the four 2-methyl-β-lactam diastereoisomers (**5b**–**8b**), only compound **5b** retained nanomolar activity ($IC_{50} = 0.24$ μM), with the other stereoisomers displaying inhibition only in the medium/high micromolar range, with a loss in potency of >2 orders of magnitude compared to **3b**. For these 2-methyl analogues of **3b** the (*S*)-configuration at α-position as well as a defined *trans* vicinal substitution (as for compound **5b**) were essential to maintain good NAAA inhibition. The insertion of a *gem*-dimethyl moiety, as for compounds **9b** and **10b**, was detrimental for activity (**9b**: $IC_{50} = 25.42$ μM; **10b**: $IC_{50} = 17.3$ μM, Table 2).

This first set of modifications of the 3-azetidinone core showed that the configuration of the two stereogenic centers and the substitution at β-position play a key role in the modulation of the potency of these analogues.

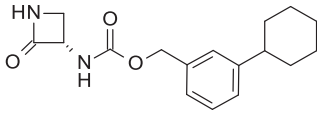
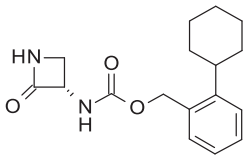
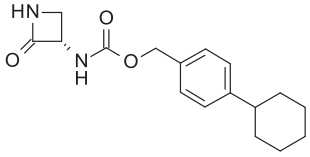
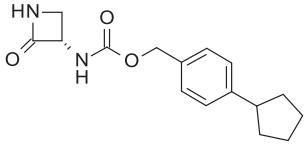
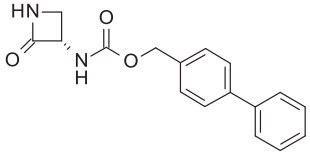
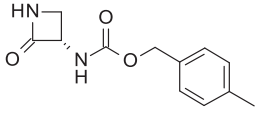
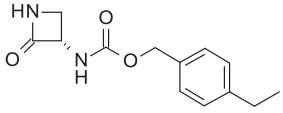
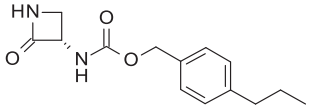
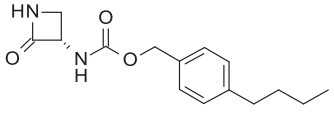
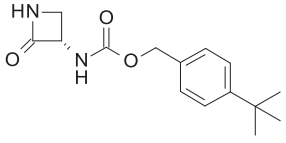
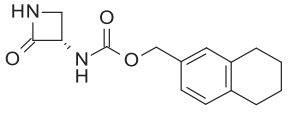
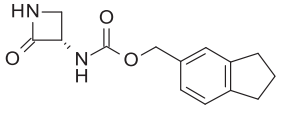
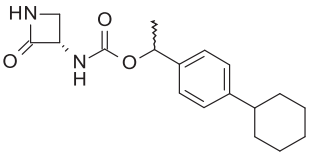
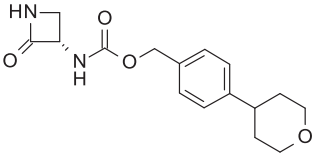
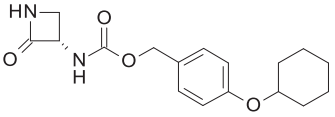
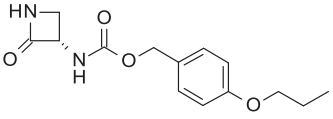
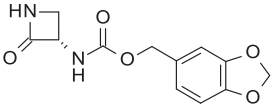
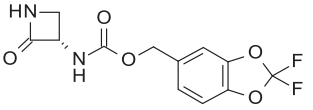
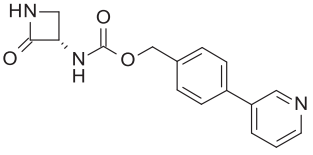
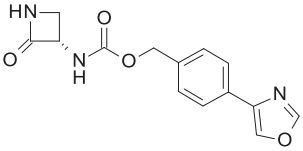
Taking into account these results and our previous findings, showing that the β-lactam core is mandatory for the activity [42], we continued the SAR expansion on β-unsubstituted β-lactam carbamic acid esters. In particular, we decided to synthesize novel analogues of compound **3b** to investigate the effects of the length and conformational flexibility of the aliphatic chain as well as of the insertion of heteroatoms (Table 3).

On the basis of the results of the SAR studies on the β-lactone carbamate [37,38] and β-lactam amide [42] classes, we learned that NAAA inhibition could be affected by varying the length of the aliphatic side chain. A limited exploration of this structural feature was carried out by decreasing (**3f**) or increasing (**3g**) the linear chain of carbamate **3b** by a methylene unit. Unexpectedly, both compounds turned out to be slightly more potent (**3f**: $IC_{50} = 0.044$ μM; **3g**: $IC_{50} = 0.049$ μM, Table 3) than **3b**, differently to what we observed in the β-lactone carbamate series [37,38].

Next, we tested whether the replacement of a carbon atom in the side chain with a heteroatom might be beneficial for both inhibitory activity and physicochemical properties. Disappointingly, the insertion of an oxygen atom in β- (**3h**), α- (**3i**) or 4'-position (**3j**) of the cyclohexyl ring was not tolerated, as all the compounds showed only micromolar activity against NAAA (**3h**: $IC_{50} = 1.28$ μM; **3i**: $IC_{50} = 2.67$ μM, **3j**: $IC_{50} = 9.51$ μM, Table 3). On the contrary a *gem*-di-fluoro moiety in *4'*-position of the cyclohexyl ring (**3k**, $IC_{50} = 0.33$ μM) was well accommodated, showing a marginal 4-fold drop in potency compared to carbamate **3b**.

Switching the 4-cyclohexylbutyl chain of **3b** into a more conformationally rigid 4'-butylcyclohexanol-derived carbamate, as in analogue **3l**, resulted only in a limited reduction of NAAA inhibition ($IC_{50} = 0.32$ μM).

Table 4
Inhibitory potencies (IC_{50}) of compounds **3u**–**am** on *h*-NAAA activity.[a]

Compounds	Structure	IC_{50} (μM) ± SD
3u		1.72 ± 0.24
3v		12.83 ± 0.21
3w		0.041 ± 0.009
3x		0.071 ± 0.011
3e		0.513 ± 0.108
3y		1.04 ± 0.26
3z		0.16 ± 0.061
3aa		0.052 ± 0.018
3ab		0.023 ± 0.002
3ac		0.31 ± 0.056
3ad		0.14 ± 0.02
3ae		0.13 ± 0.027
3af		4.47 ± 1.11
3ag		1.37 ± 0.121
3ah		2.07 ± 0.061
3ai		0.25 ± 0.049
3aj		2.19 ± 0.433
3ak		0.085 ± 0.011
3al		8.11 ± 1.99
3am		1.88 ± 0.257

[a] IC_{50} values are reported as mean values of three or more determinations.

To further evaluate the role of the terminal cyclohexyl ring, we replaced this group with either a smaller cyclopentyl residue (**3m**) or a planar phenyl ring (**3n**). Whereas a slight improvement in activity was observed for β-lactam **3m** (IC_{50} = 0.033 μM), the phenyl substituted derivative displayed a decrease in potency of ca. 15-fold (**3n**: IC_{50} = 1.11 μM). To test whether substituents on the terminal phenyl ring could recover potency, we synthesized compounds **3o**–**q** featuring moieties with different stereo-electronic properties. Although the 4-ethyl (**3o**) and 4-methoxy (**3p**) substituted phenyl derivatives turned out to be more active than the corresponding unsubstituted analogue **3n** (IC_{50} = 0.23 μM and IC_{50} = 0.52 μM, respectively), their activity was still 3- to 8-fold lower than that of the cyclohexyl substituted carbamate **3b**. Interestingly, the insertion of a 4-trifluoromethyl group (**3q**) was well tolerated, showing similar activity to **3b** (IC_{50} = 0.073 μM). This recovery of activity for compound **3q** could be explained by the more lipophilic nature of the trifluoromethyl group, which possibly

Table 5
Inhibitory potencies (IC_{50}) on *h*-NAAA and *h*-AC, % inhibitory activity (%Inhib.) on *h*-FAAH, mouse and rat plasma and microsomal stability ($t_{½}$), and solubility of compounds **3b,k,w,ab,ai,ak**.

Compounds	*h*-NAAA IC_{50} (μM) ± SD[a,d]	*h*-NAAA IC_{50} (μM) ± SD[b,d]	*h*-AC IC_{50} (μM) ± SD[c,d]	*h*-FAAH (%Inhib.)[f]	*m*-Plasma stability ($t_{½}$) min[g,j]	*m*-Metabolic stability ($t_{½}$) min[h,j,k]	*r*-Plasma stability ($t_{½}$) min[g,j]	*r*-Metabolic stability ($t_{½}$) min[h,j,k]	Kinetic solubility (PBS) (μM)[i,j]
3b	0.073 ± 0.008	0.027 ± 0.005	6.27 ± 0.83 (*230*)[e]	n.a.	41 ± 8	<5	12 ± 6	<5	144 ± 9
3k	0.337 ± 0.101	0.076 ± 0.018	10.49 ± 1.26 (*140*)[e]	n.a.	33 ± 1	34 ± 1	7 ± 2	10 ± 2	>250
3w	0.041 ± 0.014	0.012 ± 0.001	1.51 ± 0.797 (125)[e]	n.a.	57 ± 18	5 ± 1	104 ± 24	<5	51 ± 3
3ab	0.023 ± 0.02	0.007 ± 0.001	0.39 ± 0.04 (*55*)[e]	n.a.	29 ± 11	<5	>120	<5	83 ± 3
3ai	0.25 ± 0.049	0.048 ± 0.009	2.41 ± 0.53 (*50*)[e]	n.a.	31 ± 13	11 ± 1	>120	<5	>250
3ak	0.085 ± 0.012	0.022 ± 0.001	8.09 ± 2.31 (*365*)[e]	n.a.	25 ± 5	30 ± 2	>120	40 ± 4	>250

[a] *h*-NAAA fluorogenic assay.
[b] *h*-NAAA UPLC/MS assay.
[c] *h*-AC UPLC/MS assay.
[d] IC_{50} values are reported as mean values of three or more determinations.
[e] *h*-NAAA vs. *h*-AC ratio.
[f] *h*-FAAH fluorogenic assay. Inhibitory activities are reported as a % value of two determinations at 10 μM (n.a. = <10% inhibition).
[g] *Mouse* or *Rat* plasma (100% v/v), 5% DMSO, 37 °C, pH 7.4.
[h] *Mouse* or *Rat* Liver microsomes/NADPH (100% v/v), 10% DMSO, 0.1 M Tris–HCl buffer, 37 °C, pH 7.4.
[i] Kinetic solubility, PBS, pH 7.4.
[j] Stability and solubility values are reported as mean values of three or more determinations.
[k] <5 = Stability lower than first measurable point.

finds better accommodation in the enzyme hydrophobic active site. Finally, while the insertion of a nitrogen atom in the aromatic ring, as for pyridine derivative **3r**, resulted in an almost complete loss of activity (IC_{50} = 30.65 μM), rigidifying the side chain with a *E*- or *Z*-configured double bond conjugated to the phenyl ring did not affect inhibitory potency compared to the linear saturated analogue **3n** (**3s**: IC_{50} = 2.89 μM; **3t**: IC_{50} = 1.51 μM, Table 3).

Overall, the results reported in Table 3 indicated a clear preference for lipophilic, linear side chains, and highlighted a limited possibility to capture further interactions by introducing heteroatoms. These outcomes further support the idea that lipophilic moieties, which resemble the enzyme natural substrate, are preferentially accommodated in the NAAA active site.

To obtain additional insights into the SAR of this novel chemical class, we evaluated the influence of the flexibility of the aliphatic side chain on NAAA inhibition. We explored the possibility to fix the 4-cycloexylbutyl moiety of **3b** in a more rigid conformation and then we examined a series of less conformationally flexible analogues (Table 4).

First, we replaced the 4-cyclohexylbutyl moiety with a *meta*-cyclohexyl substituted benzyl carbamate, as in β-lactam **3u**. This modification turned out to be detrimental for NAAA inhibition (IC_{50} = 1.72 μM), leading to a 25-fold drop in potency compared to **3b**. To assess the effect of regiochemistry for this type of substitution, *ortho*- (**3v**) and *para*- (**3w**) regioisomers were synthesized and tested. While compound **3v** displayed inhibitory activity only in the high micromolar range (IC_{50} = 12.83 μM), the *para*-cyclohexyl benzyl carbamate **3w** exhibited a slight increase in potency (IC_{50} = 0.041 μM) with respect to the more flexible analogue **3b**. The finding that a *para*-substituted benzyl group retained a higher NAAA inhibitory activity compared to the corresponding *ortho*- and *meta*-isomers prompted us to focus our subsequent efforts on the synthesis of *para*-substituted benzyl carbamates. We evaluated the role of the ring size and type, the influence of linear alkyl substituents, and the combination of these changes with a simultaneous introduction of heteroatoms (Table 4).

While a *para*-cyclopentyl ring (**3x**) showed an IC_{50} value comparable to the *para*-cyclohexyl analogue (IC_{50} = 0.071 μM), the replacement of the cyclohexyl with a planar phenyl ring, as in **3e**, resulted in a ca. 13-fold drop in activity (IC_{50} = 0.51 μM), as previously reported (Table 1) and in agreement with the analogous reduction in NAAA inhibition observed with β-lactam **3n** versus **3b** (Table 3). These outcomes clearly indicate that the presence of a terminal unsubstituted phenyl ring in the carbamic side chain is only marginally tolerated due to either shape or conformational reasons.

The replacement of the *para*-cyclohexyl moiety of **3w** with short linear alkyl substituents (**3y**–**ab**, Table 4) led to a progressive increase in inhibitory activity by extending the length of the alkyl chain from 1 to 4 methylene units (IC_{50} = 1.04 μM-0.023 μM). In particular the *n*-butyl substituted β-lactam **3ab** displayed the highest potency (IC_{50} = 0.023 μM) within this small set of analogues. The replacement of the *n*-butyl moiety with a bulkier *t*-butyl group was not well tolerated, leading to a ca.13-fold drop in potency for β-lactam **3ac** (IC_{50} = 0.31 μM) with respect to **3ab**. These observations are consistent with our previously findings on NAAA inhibitors from different chemical classes [34,38,42], in which derivatives with linear alkyl side-chains exhibited in general good to high potency.

Then, we turned our attention to carbamates possessing fused rings, such as the bicyclic tetralinyl- (**3ad**) and indanyl-derived (**3ae**) analogues. These compounds showed quite good potency (IC_{50} = 0.14 μM and 0.13 μM, respectively), although being slightly less active than the corresponding ring-opened derivatives **3ab** and **3aa** (3- to 6-fold drop in potency).

To further map the region of the enzyme occupied by the carbamate moiety and the adjacent carbon atom, we evaluated the effect of the substitution of the benzylic position in the potent β-lactam **3w**. The introduction of a methyl group close to the carbamic function, as in **3af**, turned out to be detrimental for potency (IC_{50} = 4.47 μM). Although compound **3af** is a mixture of diastereoisomers, which could have a substantial difference in potency [38], the observed drastic drop in activity for **3af** with respect to **3w** appears to indicate a limited space in this region of the enzyme active site, at least for this type of derivatives.

After the identification of conformationally constrained derivatives with good activity, we explored the replacement of one or more carbon atoms of the carbamic side chain with an oxygen in the attempt to further improve potency and modulate physicochemical properties. Unfortunately, in analogy with the results obtained with the linear aliphatic derivatives (**3h**–**j**, Table 3), the introduction of an oxygen atom in the side chain negatively affected potency. Although compounds **3ag** and **3ah** showed a 34- to 50-fold reduction in NAAA inhibition (**3ag**: IC_{50} = 1.37 μM; **3ah**:

IC_{50} = 2.07 μM) compared to their analogue **3w**, the *para*-propoxy substituted β-lactam **3ai** showed a limited (ca. 10-fold) decrease in potency, still being in the medium-low namomolar range (IC_{50} = 0.25 μM, Table 4).

Notably, even if an evident loss in potency was observed for benzyl-1,3-dioxolane carbamate **3aj** (IC_{50} = 2.19 μM), compared to compound **3ae**, the insertion of a *gem*-difluoro moiety on the acetal carbon atom (**3ak**) restored NAAA inhibition in the low nanomolar range (IC_{50} = 0.085 μM). As seen for β-lactam **3k**, we speculate that the increased lipophilicity of the *gem*-difluoromethylene group [65] may allow establishing additional interactions within the NAAA active site, thus increasing potency, which are somehow lost with the sole introduction of polar atoms.

The replacement of the *para*-cyclohexyl moiety in compound **3w** with a 3-pyridyl (**3al**, IC_{50} = 8.1 μM) or a 4-oxazolyl (**3am**, IC_{50} = 1.88 μM) group confirmed the negative effect of hetero-aryl substitution at least in this region, as already seen for *para*-phenyl derivative **3e**.

A selection of structurally diverse β-lactam carbamic acid esters (**3b**,**k**,**w**,**ab**,**ai**,**ak**) identified in our SAR investigation was then tested for selectivity against human Fatty Acid Amide Hydrolase (*h*-FAAH), a member of the serine hydrolase family which can cleave FAEs [66], and human Acid Ceramidase (*h*-AC) [63]. The selectivity of the selected compounds versus *h*-AC was evaluated using a UPLC-MS-based assay [64], in order to measure IC_{50} values under similar experimental conditions (Table 5).

As previously reported for rat AC [8], compound **3b** showed a high selectivity also versus *h*-AC (ca. 230-fold), having an activity only in the medium micromolar range (IC_{50} = 6.27 μM). This poor affinity towards *h*-AC was also maintained by its close *gem*-difluoro-analogue **3k**, which displayed an IC_{50} of 10.49 μM. Disappointingly, the introduction of a conformationally constrained benzylic group on the carbamate moiety, favourable for *h*-NAAA inhibition, led to a good activity on *h*-AC. As a result, compounds **3w**,**ab**,**ai** revealed a modest *h*-NAAA versus *h*-AC selectivity ratio (<130-fold, Table 5). Remarkably, the difluoro-benzodioxol substituted analogue **3ak** was only a medium micromolar *h*-AC inhibitor (IC_{50} = 8.09 μM, Table 5), therefore recovering a high selectivity (>370-fold).

At the tested concentration (10 μM), none of the selected β-lactam carbamates inhibited *h*-FAAH in a significant manner (Table 5).

The physicochemical and drug-like profiles of β-lactams **3b**,**k**,**w**,**ab**,**ai**,**ak** were examined next (Table 5), evaluating their kinetic solubility in buffer (PBS, pH 7.4) and their plasma and metabolic stability in both mouse and rat plasma and liver microsomes, respectively. The carbamate derivatives **3b**, was fairly soluble (144 μM), but showed a low metabolic ($t_{½}$ < 5 min in both species) and plasma stability ($t_{½}$ = 12 min in rat; $t_{½}$ = 41 min in mouse) [8].

The *gem*-di-fluoro substitution on the cyclohexyl ring, as for **3k**, significantly improved the metabolic stability in mouse microsomes ($t_{½}$ = 34 min) compared to **3b**. This outcome would suggest the terminal cyclohexyl moiety in the side chain of **3b** as a possible soft spot for oxidative metabolism in this species.

The substitution at the para position of the benzylic carbamic acid ester chain, as for compounds **3w** and **3ab**, positively affected the rat plasma stability ($t_{½}$ = 104 min and >120 min, respectively), but negatively influenced the solubility (51 μM and 83 μM) and did not have any substantial effect on mouse plasma and on mouse and rat liver microsomal stability, which remained still quite low in both species ($t_{½}$ < 5 min). Reasonably, these data indicated an overall positive contribution of the benzylic substitution adjacent to the carbamic moiety on rat plasma stability, therefore reducing a possible hydrolytic cleavage of such electrophilic compounds in this species. The introduction of an oxygen atom in the linear alkyl chain of **3ab** improved the solubility of the resulting compound, **3ai** (>250 μM), maintained a good plasma stability in rat ($t_{½}$ >120 min), but did not ameliorate the high oxidative metabolism in both species.

The results reported above reasonably indicate that a carbamic acid side chain bearing an adjacent, substituted benzylic moiety could help improving plasma stability (especially in rat, **3k**,**ab** vs. **3b**), whereas the introduction of a *gem*-difluoro group in specific positions on the side chain could positively modulate the microsomal stability (**3k** vs. **3b**). These outcomes were somehow confirmed for β-lactam **3ak**, which altogether encompassed these structural requirements. In fact, to our gratification, this novel analogue showed excellent solubility (>250 μM) and good stability properties in rat (plasma $t_{½}$ > 120 min; micrososomal $t_{½}$ = 40 min) coupled with a good activity and selectivity profile (Table 5).

4. Conclusions

In the present work, we outline the key structural modifications that led to the identification of 4-cyclohexylbutyl-*N*-[(*S*)-2-oxoazetidin-3-yl]carbamate (**3b**), as a potent, selective and systemically active inhibitor of intracellular NAAA activity. Furthermore, we expanded our previous SAR investigation on 3-aminoazetidin-2-one derivative NAAA inhibitors. We structurally modified compound **3b** with the aim of defining the relevant chemical features needed to achieve both good NAAA inhibition and suitable physicochemical and drug-like properties. We investigated primarily the effect of the size and shape of the carbamic acid ester side chains, as well as the influence of the substitution at β-position of the 2-oxo-3-azetidinyl ring. The studies demonstrated the importance of the configuration of the stereogenic center at position 3 and the detrimental effect on potency of β-substitution on the β-lactam ring. While the modifications of the linear lipophilic chain, in terms of the insertion of heteroatoms or substitution of the terminal ring, resulted in no or only minor beneficial effects on NAAA inhibition, the replacement of the 4-cyclohexylbutyl chain of compound **3b** with substituted benzylic moieties turned out to be important to modulate the potency and the physicochemical profile. Thorough SAR explorations of the substitution patterns on the benzylic side-chains (site, type and shape of modifications, and insertion of heteroatoms) led to the discovery of **3ak**, a novel human NAAA inhibitor with a good activity and an improved stability profile with respect to **3b**. The structural diversity of the carbamic acid chain and the good physicochemical properties render compound **3ak** a promising tool, which might help to further investigate the potential of NAAA inhibitors as new therapeutic agents for the treatment of pain and inflammatory conditions. The pharmacological profile of **3ak** is currently under evaluation, and data will be reported in due course.

5. Experimental section

5.1. Chemistry

All the commercial available reagents and solvents were used as purchased from vendors without further purification. Dry solvents (THF, Et_2O, CH_2Cl_2, DMF, DMSO, MeOH) were purchased from Sigma–Aldrich. Automated column chromatography purifications were done using a Teledyne ISCO apparatus (Combi*Flash*® R_f) with pre-packed silica gel columns of different sizes (from 4 g up to 120 g). Mixtures of increasing polarity of cyclohexane and ethyl acetate (EtOAc), cyclohexane and methyl *tert*-butyl ether (MTBE) or dichloromethane and methanol (MeOH) were used as eluents. Hydrogenation reactions were performed using H–Cube® continuous hydrogenation equipment (SS–reaction line version),

employing disposable catalyst cartridges (CatCart®) preloaded with the required heterogeneous catalyst. Microwave heating was performed using Explorer®–48 positions instrument (CEM). 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 (DMSO–d_6) or deuterated chloroform ($CDCl_3$) as solvents. UPLC/MS analyses were run 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. Analyses were performed on an ACQUITY UPLC BEH C_{18} column (100 × 2.1mmID, particle size 1.7 μm) with a VanGuard BEH C_{18} pre-column (5 × 2.1 mmID, particle size 1.7 μm). Mobile phase was 10 mM NH_4OAc in H_2O at pH 5 adjusted with CH_3COOH (A) and 10 mM NH_4OAc in CH_3CN–H_2O (95:5) at pH 5.0. Electrospray ionization in positive and negative mode was applied. ESI was applied in positive and negative mode. Purifications by preparative HPLC/MS were run on a Waters Autopurification system consisting of a 3100 single quadrupole mass spectrometer equipped with an Electrospray Ionization interface and a 2998 Photodiode Array Detector. HPLC system included a 2747 sample manager, 2545 binary gradient module, system fluidic organizer and 515 HPLC pump. PDA range was 210–400 nm. Purifications were performed on a XBridge™ Prep C_{18} OBD column (100 × 19 mmID, particle size 5 μm) with a XBridge™ Prep C_{18} (10 × 19 mmID, particle size 5 μm) guard cartridge. Mobile phase was 10 mM NH_4OAc in MeCN–H_2O (95:5) at pH 5. Electrospray ionization in positive and negative mode was used. Accurate mass measurement (HMRS) was performed on a Synapt G2 quadrupole-Tof instrument (Waters, USA), equipped with an ESI ion source. All tested compounds (**3b**–**am, 4b**–**10b**) showed ≥ 95% purity by NMR and UPLC/MS analysis.

5.1.1. General procedure for the synthesis of carbamates 3b–am, 4b–10b (Schemes 1 and 4)

5.1.1.1. Synthesis of [(S)-2-oxoazetidin-3-yl]-ammonium acetate (**14**).

Off-white solid. Experimental procedure and ^{1}H NMR are according to literature [42].

5.1.1.1.1. Preparation of activated alcohols as alkyl-2-pyridyl carbonates 12b–am and alkyl-2-oxopyridine-1-carboxylates 13b–am (step 1). Under nitrogen atmosphere, to a stirred mixture of the corresponding alcohol **11b**–**am** (1.0 eq.) in dry CH_2Cl_2 (5.0 mL), DMAP (0.1 eq.), and 2-DPC (1.2 eq.) were added. The reaction mixture was stirred at room temperature for 16 h, then diluted with CH_2Cl_2 (20 mL) and sequentially washed with sat. NH_4Cl solution (25 mL), sat. $NaHCO_3$ solution (3 × 25 mL) and brine (25 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated to dryness at low pressure, as a mixture of alkyl-2-pyridyl carbonate **12b**–**am** and alkyl-2-oxopyridine-1-carboxylate **13b**–**am**. The mixture of isomers was not separated and used in the next step without any further purification.

5.1.1.1.2. Preparation of carbamates 3b–am, 4b–8b and 22b,23b (step 2). Under nitrogen atmosphere, to a suspension of **14**–**21** (1.0 eq.) in dry CH_2Cl_2 (2.0 mL), DIPEA (1.2 eq.) was added dropwise. Subsequently, the crude mixture containing the corresponding alkyl-2-oxopyridine-1-carboxylate (1.2 eq.) in dry CH_2Cl_2 (4.0 mL) was added. The reaction mixture was stirred at room temperature for 16 h, diluted with CH_2Cl_2 (10 mL), washed with sat. NH_4Cl solution (2 × 20 mL), sat. $NaHCO_3$ solution (2 × 20 mL), and the organic layer dried over Na_2SO_4 and concentrated to dryness. Purification was performed either by typical silica gel flash chromatography or preparative HPLC affording the desired β-lactam carbamates.

5.1.2. 4-Cyclohexylbutyl-N-[(S)-2-oxoazetidin-3-yl]-carbamate (3b)

Experimental procedure, ^{1}H and ^{13}C NMR are according to literature [8]. HRMS (*m/z*): $[M-H]^+$ calculated 269.1865, found 269.1873.

5.1.3. n-Octyl-N-[(S)-2-oxoazetidin-3-yl]-carbamate (3c)

*5.1.3.1. n-Octyl-2-pyridyl carbonate (**12c**) and n-octyl-2-oxopyridine-1-carboxylate (**13c**).* General Procedure (Step 1) was followed using commercially available *n*-octan-1-ol (**11c**) (0.24 mL, 1.53 mmol). A mixture (1:2 ratio) of *n*-octyl-2-pyridyl carbonate (**12c**) and *n*-octyl-2-oxopyridine-1-carboxylate (**13c**) was not separated and used in the next step without any further purification. R_t = 2.82 min. MS (ESI) *m/z*: 252 $[M-H]^+$. MS (ESI) *m/z*: 250 $[M-H]^-$.

*5.1.3.2. n-Octyl-N-[(S)-2-oxoazetidin-3-yl]-carbamate (**3c**).* General Procedure (Step 2) was followed using the previously described mixture (0.24 g), containing **13c**, and compound **14** (0.05 g, 0.34 mmol). Trituration with Et_2O afforded the pure title compound (0.035 g, 42%), as a white solid. R_t = 2.40 min; MS (ESI) *m/z*: 243 $[M-H]^+$, 260 $[M-NH_4]^+$, 265 $[M-Na]^+$. MS (ESI) *m/z*: 241 $[M-H]^-$. $[M-H]^+$ calculated: 243.1709, found 243.1712. ^{1}H NMR (DMSO-d_6): δ 7.96–7.88 (m, 1H), 7.78 (d, 1H, J = 8.8 Hz), 4.72–4.54 (m, 1H), 3.94 (t, 2H, J = 6.7 Hz), 3.37 (t, 1H, J = 5.4 Hz), 3.06 (dd, 1H, J = 5.2, 2.7 Hz), 1.62–1.44 (m, 2H), 1.36–1.21 (m, 12H), 0.86 (t, 3H, J = 7.2 Hz). ^{13}C NMR (DMSO-d_6): δ 168.20, 155.56, 64.07, 58.30, 42.60, 39.52, 31.17, 28.61, 28.58, 28.55, 25.31, 22.04, 13.91.

5.1.4. 5-Phenylpentyl-N-[(S)-2-oxoazetidin-3-yl]-carbamate (**3d**)

*5.1.4.1. 5-Phenylpentyl-2-pyridyl carbonate (**12d**) and 5-phenylpentyl-2-oxopyridine-1-carboxylate (**13d**).* General Procedure (Step 1) was followed using commercially available 5-phenyl-1-heptanol (**11d**) (0.3 mL, 1.83 mmol). A mixture (1:3.7 ratio) of 5-phenylpentyl-2-pyridyl carbonate (**12d**) and 5-phenylpentyl-2-oxopyridine-1-carboxylate (**13d**) was not separated and used in the next step without any further purification. R_t = 2.62 min. MS (ESI) *m/z*: 286 $[M-H]^+$, 308 $[M-Na]^+$, 324 $[M-K]^+$.

*5.1.4.2. 5-Phenylpentyl-N-[(S)-2-oxoazetidin-3-yl]-carbamate (**3d**).* General Procedure (Step 2) was followed using the previously described mixture (0.55 g), containing **13d**, and compound **14** (0.06 g, 0.41 mmol). Purification by preparative HPLC afforded the pure title compound (0.034 g, 30%), as a white solid. R_t = 2.23 min. MS (ESI) *m/z*: 277 $[M-H]^+$, 299 $[M-Na]^+$, 315 $[M-K]^+$. HRMS (*m/z*): $[M-H]^+$ calculated: 277.1552, found 277.1533. ^{1}H NMR (DMSO-d_6): δ 7.90 (s, 1H), 7.78 (d, 1H, J = 8.7 Hz), 7.30–7.24 (m, 2H), 7.21–7.13 (m, 3H), 4.66–4.46 (m, 1H), 3.95 (t, 2H, J = 6.4 Hz), 3.37 (t, 1H, J = 5.3 Hz), 3.06 (dd, 1H, J = 5.3, 2.8 Hz), 2.57 (t, 2H, J = 7.6 Hz), 1.63–1.52 (m, 4H), 1.37–1.27 (m, 2H). ^{13}C NMR (DMSO-d_6): δ 168.25, 155.59, 142.14, 128.27, 128.23, 125.62, 64.09, 58.33, 42.65, 35.07, 30.66, 28.45, 25.05.

5.1.5. (4-Phenylphenyl)-methyl-N-[(S)-2-oxoazetidin-3-yl]-carbamate (3e)

*5.1.5.1. (4-Phenylphenyl)-methyl-2-pyridyl carbonate (**12e**) and (4-phenylphenyl)-methyl-2-oxopyridine-1-carboxylate (**13e**).* General Procedure (Step 1) was followed using commercially available 4-phenylbenzyl alcohol (**11e**) (0.3 g, 1.63 mmol). A mixture (1:1.6 ratio) of (4-phenylphenyl)-methyl-2-pyridyl carbonate (**12e**) and (4-phenylphenyl)-methyl-2-oxopyridine-1-carboxylate (**13e**) was not separated and used in the next step without any further purification. R_t = 2.58 min. MS (ESI) *m/z*: 328 $[M-Na]^+$, 344 $[M-K]^+$.

*5.1.5.2. (4-Phenylphenyl)-methyl-N-[(S)-2-oxoazetidin-3-yl]-carbamate (**3e**).* General Procedure (Step 2) was followed using the previously described mixture (0.327 g) containing **13e** and compound **14** (0.05 g, 0.34 mmol). Filtration from the reaction medium afforded the pure title compound (0.063 g, 62%), as a white solid. R_t = 2.20 min; MS (ESI) *m/z*: 328 $[M-Na]^+$, 344 $[M-K]^+$. HRMS (*m/z*): $[M-H]^+$ calculated 297.1239, found 297.1247. ^{1}H NMR (DMSO-d_6): δ 7.99 (d, 1H, J = 8.7 Hz), 7.94 (s, 1H), 7.69–7.62 (m, 4H), 7.50–7.33 (m, 5H), 5.09 (s, 2H), 4.76–4.61 (m, 1H), 3.41 (t, 1H, J = 5.3 Hz), 3.09 (dd, 1H, J = 5.2, 2.8 Hz). ^{13}C NMR (DMSO-d_6): δ 168.10, 155.30, 143.09, 141.27, 130.99, 129.87, 128.08, 127.12, 126.47, 65.10, 58.10, 42.53.

5.1.6. 3-Cyclohexylpropyl-N-[(S)-2-oxoazetidin-3-yl]-carbamate (3f)

*5.1.6.1. 3-Cyclohexylpropyl-2-pyridyl carbonate (**12f**) and 3-cyclohexylpropyl-2-oxopyridine-1-carboxylate (**13f**).* General Procedure (Step 1) was followed using commercially available 3-cyclohexylpropan-1-ol (**11f**) (0.25 g, 1.76 mmol). A mixture (1:1.6 ratio) of 3-cyclohexylpropyl-2-pyridyl carbonate (**12f**) and 5-cyclohexylpropyl-2-oxopyridine-1-carboxylate (**13f**) was not separated and used in the next step without any further purification. R_t = 2.81 min. MS (ESI) *m/z*: 264 $[M-H]^+$, 286 $[M-Na]^+$, 302 $[M-K]^+$.

*5.1.6.2. 3-Cyclohexylpropyl-N-[(S)-2-oxoazetidin-3-yl]-carbamate (**3f**).* General Procedure (Step 2) was followed using the previously described mixture (0.25 g) containing **13f** and compound **14** (0.05 g, 0.34 mmol). Purification by typical silica gel flash chromatography (CH_2Cl_2/MeOH, from 100:00 to 96:4) afforded the pure title compound (0.064 g, 74%), as a white solid. R_t = 2.36 min; MS (ESI) *m/z*: 255 $[M-H]^+$, 277 $[M-Na]^+$, 293 $[M-K]^+$. HRMS (*m/z*): $[M-H]^+$ calculated 255.1709, found 255.1707. ^{1}H NMR (DMSO-d_6): δ 7.90 (s, 1H), 7.78 (d, 1H, J = 8.7 Hz), 4.66–4.48 (m, 1H), 3.93 (t, 2H, J = 6.7 Hz), 3.37 (t, 1H, J = 5.1 Hz), 3.06 (dd, 1H, J = 5.1, 2.8 Hz), 1.72–1.48 (m, 7H), 1.26–1.04 (m, 6H), 0.93–0.77 (m, 2H). ^{13}C NMR (DMSO-d_6): δ 168.21, 155.56, 64.42, 58.30, 42.60, 36.70, 32.98, 32.74, 26.13, 25.94, 25.77.

5.1.7. 5-Cyclohexylpentyl-N-[(S)-2-oxoazetidin-3-yl]-carbamate (3g)

*5.1.7.1. 5-Cyclohexylpentyl-2-pyridyl carbonate (**12g**) and 5-cyclohexylpentyl-2-oxopyridine-1-carboxylate (**13g**).* General Procedure (Step 1) was followed using 5-cyclohexylpentan-1-ol (**11g**) (0.55 g, 3.23 mmol). A mixture (1:1.9 ratio) of 5-cyclohexylpentyl-2-pyridyl carbonate (**12g**) and 5-cyclohexylpentyl-2-oxopyridine-1-carboxylate (**13g**) was not separated and used in the next step without any further purification. R_t = 3.23 min; MS (ESI) *m/z*: 292 $[M-H]^+$, 314 $[M-Na]^+$, 330 $[M-K]^+$; (ESI) *m/z*: 290 $[M-H]^-$.

*5.1.7.2. 5-Cyclohexylpentyl-N-[(S)-2-oxoazetidin-3-yl]-carbamate (**3g**).* General Procedure (Step 2) was followed using the previously described mixture (0.29 g) containing **13g** and compound **14**. Purification by typical silica gel flash chromatography (cyclohexane/EtOAc, from 100:0 to 30:70) afforded the pure title compound (0.015 g, 16%), as a white solid. R_t = 2.83 min; MS (ESI) *m/z*: 292 $[M-H]^+$, 314 $[M-Na]^+$, 330 $[M-K]^+$. HRMS (*m/z*): $[M-H]^+$ calculated: 283.2022, found 283.2025. ^{1}H NMR (DMSO-d_6): δ 7.90 (s, 1H), 7.77 (d, 1H, J = 8.7 Hz), 4.64–4.60 (m, 1H), 3.94 (t, 2H, J = 6.6 Hz), 3.37 (t, 1H, J = 5.4 Hz), 3.07 (dd, 1H, J = 5.4, 2.7 Hz), 1.71–1.60 (m, 4H), 1.55 (dt, 2H, J = 13.4, 8.1 Hz), 1.37–1.07 (m, 11H), 0.86–0.80 (m, 2H). ^{13}C NMR (DMSO-d_6): δ 168.20, 155.56, 64.07, 58.30, 42.61, 36.93, 36.83, 32.85, 28.59, 26.19, 25.90, 25.83, 25.63.

5.1.8. 2-(Cyclohexylmethoxy)-ethyl-N-[(S)-2-oxoazetidin-3-yl]-carbamate (3h)

*5.1.8.1. 2-(Cyclohexylmethoxy)-ethyl-2-pyridyl carbonate (**12h**) and 2-(cyclohexylmethoxy)-ethyl-2-oxopyridine-1-carboxylate (**13h**).* General Procedure (Step 1) was followed using 2-(cyclohexylmethoxy)-ethanol (**11h**) (0.25 g, 1.58 mmol). A mixture (1:2.1 ratio) of 2-(cyclohexylmethoxy)-ethyl-2-pyridyl carbonate (**12h**) and 2-(cyclohexylmethoxy)-ethyl-2-oxopyridine-1-carboxylate (**13h**) was not separated and used in the next step without any further purification. R_t = 1.00 min. MS (ESI) *m/z*: 280 $[M-H]^+$, 302 $[M-Na]^+$, 318 $[M-K]^+$.

*5.1.8.2. 2-(Cyclohexylmethoxy)-ethyl-N-[(S)-2-oxoazetidin-3-yl]-carbamate (**3h**).* General Procedure (Step 2) was followed using the previously described mixture (0.29 g) containing **13h** and compound **14** (0.05 g, 0.34 mmol). Purification by typical silica gel flash chromatography (cyclohexane/EtOAc from 50:50 to 25:75) afforded the pure title compound (0.067 g, 73%), as a white solid. R_t = 2.02 min; MS (ESI) *m/z*: 271 $[M-H]^+$, 293 $[M-Na]^+$, 309 $[M-K]^+$. HRMS (*m/z*): $[M-H]^+$ calculated: 271.1658, found 271.1657. ^{1}H NMR (DMSO-d_6): δ 7.93–7.86 (m, 2H), 4.63 (ddd, 1H, J = 8.4, 5.5, 2.8 Hz), 4.12–4.01 (m, 2H), 3.52 (t, 2H, J = 4.7 Hz), 3.38 (t, 1H, J = 5.5 Hz), 3.20 (d, 2H, J = 6.4 Hz), 3.07 (dd, 1H, J = 5.5, 2.8 Hz), 1.73–1.57 (m, 5H), 1.56–1.43 (m, 1H), 1.28–1.05 (m, 3H), 0.95–0.82 (m, 2H). ^{13}C NMR (DMSO-d_6): δ 168.13, 155.42, 75.96, 68.55, 63.50, 58.32, 42.56, 37.43, 29.46, 26.14, 25.32.

5.1.9. 3-(Cyclohexoxy)-propyl-N-[(S)-2-oxoazetidin-3-yl]-carbamate (3i)

*5.1.9.1. 3-(Cyclohexoxy)-propyl-2-pyridyl carbonate (**12i**) and 3-(cyclohexoxy)-propyl-2-oxopyridine-1-carboxylate (**13i**).* General Procedure (Step 1) was followed using 3-(cyclohexoxy)-propan-1-ol (**11i**) (0.15 g, 0.95 mmol). A mixture (1:1.7 ratio) of 3-(cyclohexoxy)-propyl-2-pyridyl carbonate (**12i**) and 3-(cyclohexoxy)-propyl-2-oxopyridine-1-carboxylate (**13i**) was not separated and used in the next step without any further purification. R_t = 2.30 min. MS (ESI) *m/z*: 280 $[M-H]^+$, 302 $[M-Na]^+$, 318 $[M-K]^+$.

*5.1.9.2. 3-(Cyclohexoxy)-propyl-N-[(S)-2-oxoazetidin-3-yl]-carbamate (**3i**).* General Procedure (Step 2) was followed using the previously described mixture (0.196 g) containing **13i** and compound **14** (0.038 g, 0.26 mmol). Purification by typical silica gel flash chromatography (CH_2Cl_2/MeOH, from 100:0 to 95:5) afforded the pure title compound (0.025 g, 36%), as a white solid. R_t = 1.84 min; MS (ESI) *m/z*: 271 $[M-H]^+$, 293 $[M-Na]^+$, 309 $[M-K]^+$. HRMS (*m/z*): $[M-H]^+$ calculated: 271.1658, found 271.1652. ^{1}H NMR (DMSO-d_6): δ 7.90 (s, 1H), 7.80 (d, 1H, J = 8.7 Hz), 4.66–4.50 (m, 1H), 4.01 (t, 2H, J = 6.4 Hz), 3.43 (t, 2H, J = 6.4 Hz), 3.37 (t, 1H, J = 5.5 Hz), 3.25–3.16 (m, 1H), 3.07 (dd, 1H, J = 5.2, 2.8 Hz), 1.85–1.70 (m, 4H), 1.69–1.58 (m, 2H), 1.51–1.41 (m, 1H), 1.28–1.11 (m, 5H). ^{13}C NMR (DMSO-d_6): δ 168.21, 155.56, 76.28, 63.41, 61.55, 60.48, 42.60, 31.71, 29.41, 25.38, 23.38.

5.1.10. 4-Tetrahydropyran-4-yl-butyl-N-[(S)-2-oxoazetidin-3-yl]-carbamate (3j)

*5.1.10.1. 4-Tetrahydropyran-4-yl-butyl-2-pyridyl carbonate (**12j**) and 4-tetrahydropyran-4-yl-butyl-2-oxopyridine-1-carboxylate (**13j**).* General Procedure (Step 1) was followed using 4-tetrahydropyran-4-yl-butan-1-ol (**11j**) (0.18 g, 1.14 mmol). A mixture (1:1.7 ratio) of 4-tetrahydropyran-4-yl-butyl-2-pyridyl carbonate (**12j**) and 4-tetrahydropyran-4-yl-butyl-2-oxopyridine-1-carboxylate (**13j**) was not separated and used in the next step without any further purification. R_t = 1.99 min. MS (ESI) *m/z*: 280 $[M-H]^+$, 302 $[M-Na]^+$, 318 $[M-K]^+$.

*5.1.10.2. 4-Tetrahydropyran-4-yl-butyl-N-[(S)-2-oxoazetidin-3-yl]-carbamate (**3j**).* General Procedure (Step 2) was followed using the previously described mixture (0.196 g) containing **13j** and compound **14** (0.05 g, 0.34 mmol). Purification by typical silica gel flash chromatography (CH_2Cl_2/MeOH, from 100:0 to 95:5) afforded the pure title compound (0.023 g, 25%), as a white solid. $R_t = 1.61$ min; MS (ESI) *m/z*: 271 $[M-H]^+$, 293 $[M-Na]^+$, 309 $[M-K]^+$. HRMS (*m/z*): $[M-H]^+$ calculated 271.1658, found 271.1661. ^{1}H NMR (DMSO-d_6): δ 7.90 (s, 1H), 7.78 (d, 1H, $J = 8.9$ Hz), 4.66–4.49 (m, 1H), 3.95 (t, 2H, $J = 6.6$ Hz), 3.81 (dd, 2H, $J = 11.5$, 4.3 Hz), 3.37 (t, 1H, $J = 5.3$ Hz), 3.25 (td, 2H, $J = 11.5$, 2.9 Hz), 3.06 (dd, 1H, $J = 5.2$, 2.8 Hz), 1.59–1.04 (m, 11H). ^{13}C NMR (DMSO-d_6): δ 168.22, 155.57, 67.08, 64.07, 58.31, 42.62, 36.00, 34.25, 32.73, 28.72, 22.13.

5.1.11. 4-(4,4-Difluorocyclohexyl)-butyl-N-[(S)-2-oxoazetidin-3-yl]-carbamate (3k)

*5.1.11.1. (4,4-Difluorocyclohexyl)-butyl-2-pyridyl carbonate (**12k**) and (4,4-difluorocyclohexyl)-butyl-2-oxopyridine-1-carboxylate (**13k**).* General Procedure (Step 1) was followed using (4,4-difluorocyclohexyl)-butan-1-ol (**11k**) (0.12 g, 0.63 mmol). A mixture (1:2.5 ratio) of (4,4-difluorocyclohexyl)-butyl-2-oxopyridine-1-carboxylate (**12k**) and (4,4-difluorocyclohexyl)-butyl-2-pyridyl carbonate (**13k**) was not separated and used in the next step without any further purification.

*5.1.11.2. (4,4-Difluorocyclohexyl)-butyl-N-[(S)-2-oxoazetidin-3-yl]-carbamate (**3k**).* General Procedure (Step 2) was followed using the previously described mixture (0.22 g) containing **13k** and compound **14** (0.03 g, 0.21 mmol). Purification by preparative HPLC afforded the pure title compound (0.028 g, 44%), as white solid. $R_t = 2.21$ min; MS (ESI) *m/z*: 305 $[M-H]^+$, 327 $[M-Na]^+$, 343 $[M-K]^+$. HRMS (*m/z*): $[M-H]^+$ calculated: 305.1677, found 305.1680. ^{1}H NMR (DMSO-d_6): δ 7.90 (s, 1H), 7.78 (d, 1H, $J = 8.8$ Hz), 4.65–4.50 (m, 1H), 4.00–3.90 (m, 2H), 3.37 (t, 1H, $J = 5.4$ Hz), 3.06 (dd, 1H, $J = 5.2$, 2.8 Hz), 2.03–1.90 (m, 2H), 1.85–1.66 (m, 4H), 1.57–1.48 (m, 2H), 1.41–1.03 (m, 7H). ^{13}C NMR (DMSO-d_6): δ 168.22, 155.57, 64.04, 58.31, 42.62, 34.57, 32.84, 32.81, 28.59, 28.49, 22.88.

5.1.12. (1s,4R) and (1r,4S)-(4-butylcyclohexyl)-N-[(S)-2-oxoazetidin-3-yl]-carbamate (3l)

*5.1.12.1. (1s,4R) and (1r,4S)-(4-butylcyclohexyl)-2-pyridyl carbonate (**12l**) and (1s,4R) and (1r,4S)-(4-butylcyclohexyl)-2-oxopyridine-1-carboxylate (**13l**).* General Procedure (Step 1) was followed using a stereoisomeric mixture of commercially available 4-butylcyclohexanol (**11l**) (0.275 mL, 1.60 mmol). A mixture (1:1.7 ratio) of (1*s*,4*R*) and (1*r*,4*S*)-(4-butylcyclohexyl)-2-pyridyl carbonate (**12l**) and (1*s*,4*R*) and (1*r*,4*S*)-(4-butylcyclohexyl)-2-oxopyridine-1-carboxylate (**13l**) was not separated and used in the next step without any further purification. $R_t = 3.01$ min; MS (ESI) *m/z*: 278 $[M-H]^+$, 300 $[M-Na]^+$.

*5.1.12.2. (1s,4R) and (1r,4S)-(4-butylcyclohexyl)-N-[(S)-2-oxoazetidin-3-yl]-carbamate (**3l**).* General Procedure (Step 2) was followed using the previously described mixture (0.22 g) containing **13l** and compound **14** (0.042 g, 0.29 mmol). Purification by typical silica gel column chromatography (cyclohexane/EtOAc 85:15) afforded the title compound (0.06 g, 79%), as a mixture (1:1 ratio) of two diastereoisomers, as a white solid. *Isomer 1*: $R_t = 2.50$ min; MS (ESI) *m/z*: 269 $[M-H]^+$, 291 $[M-Na]^+$ *Isomer 2*: $R_t = 2.57$ min; MS (ESI) *m/z*: 269 $[M-H]^+$, 291 $[M-Na]^+$. HRMS (*m/z*): $[M-H]^+$ calculated: 269.1865, found 269.1866/269.1865. ^{1}H NMR (DMSO-d_6): δ 7.93–7.88 (m, 2H), 7.74 (d, 2H, $J = 8.9$ Hz), 4.76–4.71 (m, 1H), 4.69–4.59 (m, 2H), 4.42 (tt, 1H, $J = 11.0$, 4.2 Hz), 3.38 (td, 2H, $J = 5.4$, 1.7 Hz), 3.17–3.02 (m, 2H), 1.96–1.86 (m, 2H), 1.79–1.70 (m, 4H), 1.55–1.44 (m, 3H), 1.34–1.13 (m, 18H), 1.05–0.83 (m, 9H). ^{13}C NMR (DMSO-d_6): δ 168.27, 155.01, 73.14, 69.50, 58.25, 42.61, 35.66, 31.65, 30.53, 29.19, 22.35, 13.94.

5.1.13. 4-Cyclopentylbutyl-N-[(S)-2-oxoazetidin-3-yl]-carbamate (3m)

*5.1.13.1. 4-Cyclopentylbutyl-2-pyridyl carbonate (**12m**) and 4-cyclopentylbutyl 2-oxopyridine-1-carboxylate (**13m**).* General Procedure (Step 1) was followed using 4-cyclopentylbutan-1-ol (**11m**) (0.41 g, 2.89 mmol). A mixture (1:1.7 ratio) of 4-cyclopentylbutyl-2-pyridyl carbonate (**12m**) and 4-cyclopentylbutyl 2-oxopyridine-1-carboxylate (**13m**) was not separated and used in the next step without any further purification. $R_t = 2.84$ min. MS (ESI) *m/z*: 264 $[M-H]^+$, 286 $[M-Na]^+$, 302 $[M-K]^+$.

*5.1.13.2. 4-Cyclopentylbutyl-N-[(S)-2-oxoazetidin-3-yl]-carbamate (**3m**).* General Procedure (Step 2) was followed using the previously described mixture (0.24 g) containing **13m** and compound **14** (0.05 g, 0.34 mmol). Trituration with Et_2O afforded the pure title compound (0.032 g, 37%), as a white solid. $R_t = 2.42$ min; MS (ESI) *m/z*: 255 $[M-H]^+$, 277 $[M-Na]^+$, 293 $[M-K]^+$. HRMS (*m/z*): $[M-H]^+$ calculated 255.1709, found 255.1708. ^{1}H NMR (DMSO-d_6): δ 7.90 (s, 1H), 7.78 (d, 1H, $J = 8.7$ Hz), 4.65–4.58 (m, 1H), 3.95 (t, 2H, $J = 6.7$ Hz), 3.37 (t, 1H, $J = 5.2$ Hz), 3.06 (dd, 1H, $J = 5.2$, 2.8 Hz), 1.77–1.65 (m, 3H), 1.60–1.42 (m, 6H), 1.35–1.22 (m, 4H), 1.10–0.97 (m, 2H). ^{13}C NMR (DMSO-d_6): δ 168.21, 155.57, 64.09, 58.30, 42.61, 39.55, 35.23, 32.16, 28.82, 24.68, 24.53.

5.1.14. 4-Phenylbutyl-N-[(3S)-2-oxoazetidin-3-yl]-carbamate (3n)

*5.1.14.1. 4-Phenylbutyl-2-pyridyl carbonate (**12n**) and 4-phenylbutyl-2-oxopyridine-1-carboxylate (**13n**).* General Procedure (Step 1) was followed using commercially available 4-phenylbutan-1-ol (**11n**) (0.3 mL, 2.0 mmol). A mixture (1:1.8 ratio) of 4-phenylbutyl-2-pyridyl carbonate (**12n**) and 4-phenylbutyl-2-oxopyridine-1-carboxylate (**13n**) was not separated and used in the next step without any further purification. $R_t = 2.43$ min. MS (ESI) *m/z*: 272 $[M-H]^+$, 294 $[M-Na]^+$, 310 $[M-K]^+$.

*5.1.14.2. 4-Phenylbutyl-N-[(S)-2-oxoazetidin-3-yl]-carbamate (**3n**).* General Procedure (Step 2) was followed using the previously described mixture (0.258 g) containing **13n** and compound **14** (0.05 g, 0.34 mmol). Purification by typical silica gel flash chromatography (CH_2Cl_2/MeOH, from 100:00 to 97:3) afforded the pure title compound (0.071 g, 80%), as a white solid. $R_t = 2.03$ min; MS (ESI) *m/z*: 263 $[M-H]^+$, 285 $[M-Na]^+$, 302 $[M-K]^+$. HRMS (*m/z*): 263.1394 $[M-H]^+$ calculated 263.1396, found 263.1394. ^{1}H NMR (DMSO-d_6): δ 7.90 (s, 1H), 7.79 (d, 1H, $J = 8.8$ Hz), 7.31–7.24 (m, 2H), 7.22–7.15 (m, 3H), 4.66–4.50 (m, 1H), 3.98 (t, 2H, $J = 5.8$ Hz), 3.38 (t, 1H, $J = 5.4$ Hz), 3.06 (dd, 1H, $J = 5.1$, 2.8 Hz), 2.59 (t, 2H, $J = 7.4$ Hz), 1.67–1.50 (m, 4H). ^{13}C NMR (DMSO-d_6): δ 168.21, 155.56, 141.92, 128.26, 128.23, 125.67, 63.91, 58.31, 42.63, 34.67, 28.17, 27.30.

5.1.15. 4-(4-Ethylphenyl)-butyl-N-[(S)-2-oxoazetidin-3-yl]-carbamate (3o)

*5.1.15.1. 4-(4-Ethylphenyl)-butyl-2-pyridyl carbonate (**12o**) and 4-(4-ethylphenyl)-butyl-2-oxopyridine-1-carboxylate (**13o**).* General Procedure (Step 1) was followed using 4-(4-ethylphenyl)-butan-1-ol (**11o**) (0.36 g, 2.04 mmol). A mixture (1:1.5 ratio) of 4-(4-ethylphenyl)-butyl-2-pyridyl carbonate (**12o**) and 4-(4-ethylphenyl)-butyl-2-oxopyridine-1-carboxylate (**13o**) was not separated and used in the next step without any further purification. $R_t = 2.79$ min. MS (ESI) *m/z*: 300 $[M-H]^+$, 322 $[M-Na]^+$, 338 $[M-K]^+$; MS (ESI) *m/z*: 298 $[M-H]^-$.

*5.1.15.2. 4-(4-Ethylphenyl)-butyl-N-[(S)-2-oxoazetidin-3-yl]-carbamate (**3o**).* General Procedure (Step 2) was followed using the

previous described mixture (0.258 g) containing **13o** and compound **14** (0.06 g, 0.41 mmol). Purification by typical silica gel flash chromatography (cyclohexane/AcOEt, from 90:10 to 40:60) afforded the pure title compound (0.061 g, 51%), as a white solid. R_t = 2.42 min; MS (ESI) *m/z*: 291 $[M-H]^+$, 308 $[M-NH_4]^+$, 329 $[M-K]^+$. HRMS (*m/z*): $[M-H]^+$ calculated: 291.1709, found 291.1708. ^{1}H NMR (DMSO-d_6): δ 7.90 (s, 1H), 7.79 (d, 1H, J = 8.7 Hz), 7.05–7.02 (m, 4H), 4.64–4.62 (m, 1H), 3.97 (t, 2H, J = 5.8 Hz), 3.37 (t, 1H, J = 5.2 Hz), 3.06 (dd, 1H, J = 5.2, 2.7 Hz), 2.60–2.50 (m, 4H), 1.75–1.62 (m, 4H), 1.15 (t, 3H, J = 7.6 Hz). ^{13}C NMR (DMSO-d_6): δ 168.17, 155.53, 140.94, 139.05, 128.16, 127.59, 63.90, 58.30, 42.62, 34.25, 28.17, 27.72, 27.32, 15.65.

5.1.16. 4-(4-Methoxyphenyl)-butyl-N-[(S)-2-oxoazetidin-3-yl]-carbamate (3p)

*5.1.16.1. 4-(4-Methoxyphenyl)-butyl-2-pyridyl carbonate (**12p**) and 4-(4-methoxyphenyl)-butyl-2-oxopyridine-1-carboxylate (**13p**).* General Procedure (Step 1) was followed using commercially available 4-methoxyphenylbutanol (**11p**) (0.3 g, 1.67 mmol). A mixture (1:1.7 ratio) of 4-(4-methoxyphenyl)-butyl-2-pyridyl carbonate (**12p**) and 4-(4-methoxyphenyl)-butyl-2-oxopyridine-1-carboxylate (**13p**) was not separated and used in the next step without any further purification. R_t = 2.40 min. MS (ESI) *m/z*: 302 $[M-H]^+$, 324 $[M-Na]^+$, 340 $[M-K]^+$.

*5.1.16.2. 4-(4-Methoxyphenyl)-butyl-N-[(S)-2-oxoazetidin-3-yl]-carbamate (**3p**).* General Procedure (Step 2) was followed using the previously described mixture (0.276 g) containing **13p** and compound **14** (0.05 g, 0.34 mmol). Purification by typical silica gel flash chromatography (CH_2Cl_2/MeOH, from 100:0 to 95:5) afforded the pure title compound (0.075 g, 76%), as a white solid. R_t = 2.02 min; MS (ESI) *m/z*: 293 $[M-H]^+$, 315 $[M-Na]^+$, 331 $[M-K]^+$. HRMS (*m/z*): $[M-H]^+$ calculated: 293.1501, found 293.1498. ^{1}H NMR (DMSO-d_6): δ 7.90 (s, 1H), 7.79 (d, 1H, J = 8.8 Hz), 7.13–7.06 (m, 2H), 6.86–6.80 (m, 2H), 4.66–4.49 (m, 1H), 3.97 (t, 2H, J = 5.7 Hz), 3.71 (s, 3H), 3.37 (t, 1H, J = 5.2 Hz), 3.06 (dd, 1H, J = 5.2, 2.6 Hz), 2.55–2.50 (m, 2H), 1.60–1.49 (m, 4H). ^{13}C NMR (DMSO-d_6): δ 168.20, 157.35, 155.55, 133.76, 129.16, 113.66, 63.93, 58.30, 54.93, 42.63, 33.76, 28.11, 27.50.

5.1.17. 4-[4-(Trifluoromethyl)-phenyl]-butyl-N-[(S)-2-oxoazetidin-3-yl]-carbamate (3q)

*5.1.17.1. 4-[4-(Trifluoromethyl)-phenyl]-butyl-2-pyridyl carbonate (**12q**) and 4-[4-(trifluoromethyl)-phenyl]-butyl-2-oxopyridine-1-carboxylate (**13q**).* General Procedure (Step 1) was followed using 4-[4-(trifluoromethyl)-phenyl]-butan-1-ol (**11q**) (0.25 g, 1.14 mmol). A mixture (1:1.5 ratio) of 4-[4-(trifluoromethyl)-phenyl]-butyl-2-pyridyl carbonate (**12q**) and 4-[4-(trifluoromethyl)-phenyl]-butyl-2-oxopyridine-1-carboxylate (**13q**) was not separated and used in the next step without any further purification. R_t = 2.70 min. MS (ESI) *m/z*: 340 $[M-H]^+$, 362 $[M-Na]^+$, 378 $[M-K]^+$; MS (ESI) *m/z*: 338 $[M-H]^-$.

*5.1.17.2. 4-[4-(Trifluoromethyl)-phenyl]-butyl-N-[(S)-2-oxoazetidin-3-yl]-carbamate (**3q**).* General Procedure (Step 2) was followed using the previous described mixture (0.276 g) containing **13q** and compound **14** (0.06 g, 0.41 mmol). Purification by typical silica gel flash chromatography (cyclohexane/EtOAc, from 90:10 to 40:60) afforded the pure title compound (0.071 g, 52%), as a white solid. R_t = 2.35 min; MS (ESI) *m/z*: 331 $[M-H]^+$, 348 $[M-NH_4]^+$, 353 $[M-Na]^+$, 369 $[M-K]^+$. HRMS (*m/z*): $[M-H]^+$ calculated 331.127, found 331.1276. ^{1}H NMR (DMSO-d_6): δ 7.91 (s, 1H), 7.80 (d, 1H, J = 8.7 Hz), 7.63 (d, 2H, J = 8.0 Hz), 7.43 (d, 2H, J = 8.0 Hz), 4.64–4.60 (m, 1H), 3.99 (t, 2H, J = 6.1 Hz), 3.37 (t, 1H, J = 5.2), 3.06 (dd, 1H, J = 5.2, 2.7 Hz), 2.69 (t, 2H, J = 7.3 Hz), 1.73–1.48 (m, 4H). ^{13}C NMR (DMSO-d_6): δ 168.18, 155.52, 146.97, 126.55, 125.08, 123.09, 63.80, 58.30, 42.62, 34.35, 28.08, 26.92.

5.1.18. 4-(2-Pyridyl)-butyl-N-[(S)-2-oxoazetidin-3-yl]-carbamate (3r)

*5.1.18.1. 4-(2-Pyridyl)-butyl-2-pyridyl carbonate (**12r**) and 4-(2-pyridyl)-butyl-2-oxopyridine-1-carboxylate (**13r**).* General Procedure (Step 1) was followed using commercially available 4-(2-pyridyl)-butan-1-ol (**11r**) (0.30 g, 1.98 mmol). A mixture (1:1.5 ratio) of 4-(2-pyridyl)-butyl-2-pyridyl carbonate (**12r**) and 4-(2-pyridyl)-butyl-2-oxopyridine-1-carboxylate (**13r**) was not separated and used in the next step without any further purification. R_t = 1.64 min. MS (ESI) *m/z*: 273 $[M-H]^+$, 295 $[M-Na]^+$, 311 $[M-K]^+$; MS (ESI) *m/z*: 271 $[M-H]^-$

*5.1.18.2. 4-(2-Pyridyl)-butyl-N-[(S)-2-oxoazetidin-3-yl]-carbamate (**3r**).* General Procedure (Step 2) was followed using the previously described mixture (0.18 g) containing **13r** and compound **14** (0.05 g, 0.34 mmol). Purification by typical silica gel flash chromatography using a Teledyne ISCO apparatus (cyclohexane/AcOEt, from 90:10 to 10:90) followed by preparative HPLC afforded the pure title compound (0.02 g, 22%), as a white solid. R_t = 1.28 min; MS (ESI) *m/z*: 264 $[M-H]^+$, 286 $[M-Na]^+$, 302 $[M-K]^+$. HRMS (*m/z*): $[M-H]^+$ calculated: 264.1348, found 264.1350. ^{1}H NMR (DMSO-d_6): δ 8.50–8.44 (m, 1H), 7.90 (s, 1H), 7.79 (d, 1H, J = 8.7 Hz), 7.68 (td, 1H, J = 7.6, 1.9 Hz), 7.24 (d, 1H, J = 7.6 Hz), 7.18 (ddd, 1H, J = 7.6, 4.9, 0.8 Hz), 4.69–4.62 (m, 1H), 3.98 (t, 2H, J = 6.5 Hz), 3.37 (t, 1H, J = 5.3 Hz), 3.06 (dd, 1H, J = 5.3, 2.7 Hz), 2.74 (t, 2H, J = 7.6 Hz), 1.71 (quint, 2H, J = 8.0, 7.5 Hz), 1.62–1.52 (m, 2H). ^{13}C NMR (DMSO-d_6): δ 168.26, 155.58, 149.61, 147.16, 137.33, 135.83, 123.46, 63.87, 58.32, 42.69, 31.68, 28.12, 27.09.

5.1.19. [(E)-4-phenylbut-3-enyl]-N-[(S)-2-oxoazetidin-3-yl]-carbamate (3s)

*5.1.19.1. [(E)-4-phenylbut-3-enyl]-2-pyridyl carbonate (**12s**) and [(E)-4-phenylbut-3-enyl]-2-oxopyridine-1-carboxylate (**13s**).* General Procedure (Step 1) was followed using (*E*)-non-3-en-1-ol (**11s**) (0.21 g, 1.40 mmol). A mixture (1:2.2 ratio) of [(*E*)-4-phenylbut-3-enyl]-2-pyridyl carbonate (**12s**) and [(*E*)-4-phenylbut-3-enyl]-2-oxopyridine-1-carboxylate (**13s**) was not separated and used in the next step without any further purification. R_t = 2.33 min. MS (ESI) *m/z*: 270 $[M-H]^+$, 292 $[M-Na]^+$, 308 $[M-K]^+$.

*5.1.19.2. [(E)-4-phenylbut-3-enyl]-N-[(S)-2-oxoazetidin-3-yl]-carbamate (**3s**).* General Procedure (Step 2) was followed using the previously described mixture (0.29 g) containing **13s** and compound **14** (0.05 g, 0.34 mmol). Purification by preparative HPLC afforded the pure title compound (0.034 g, 40%), as a white solid. R_t = 1.96 min; MS (ESI) *m/z*: 261 $[M-H]^+$, 283 $[M-Na]^+$, 299 $[M-K]^+$. HRMS (*m/z*): $[M-Na]^+$ calculated 283.1059, found 283.1065. ^{1}H NMR (DMSO-d_6): δ 7.91 (s, 1H), 7.85 (d, 1H, J = 8.9 Hz), 7.38 (d, 2H, J = 7.6 Hz), 7.31 (t, 2H, J = 7.6 Hz), 7.24–7.18 (m, 1H), 6.48 (d, 1H, J = 16.0 Hz), 6.32–6.21 (m, 1H), 4.68–4.50 (m, 1H), 4.09 (t, 2H, J = 6.5 Hz), 3.36 (t, 1H, J = 5.2 Hz), 3.06 (dd, 1H, J = 5.2, 2.7 Hz), 2.47 (t, 2H, J = 6.5 Hz). ^{13}C NMR (DMSO-d_6): δ 168.13, 155.56, 131.61, 128.52, 127.14, 126.34, 125.88, 63.45, 58.31, 42.59, 32.28.

5.1.20. [(Z)-4-phenylbut-3-enyl]-N-[(S)-2-oxoazetidin-3-yl]-carbamate (3t)

*5.1.20.1. [(Z)-4-phenylbut-3-enyl]-2-pyridyl carbonate (**12t**) and [(Z)-4-phenylbut-3-enyl]-2-oxopyridine-1-carboxylate (**13t**).* General Procedure (Step 1) was followed using (*Z*)-non-3-en-1-ol (**11t**) (0.375 g, 2.53 mmol). A mixture (1:1.9 ratio) of [(*Z*)-4-phenylbut-3-enyl]-2-pyridyl carbonate (**12t**) and [(*Z*)-4-phenylbut-3-enyl]-2-oxopyridine-1-carboxylate (**13t**) was not

separated and used in the next step without any further purification. $R_t = 2.33$ min. MS (ESI) *m/z*: 270 $[M-H]^+$, 292 $[M-Na]^+$, 308 $[M-K]^+$.

*5.1.20.2. [(Z)-4-phenylbut-3-enyl]-N-[(S)-2-oxoazetidin-3-yl]-carbamate (**3t**).* General Procedure (Step 2) was followed using the previously described mixture (0.29 g) containing **13t** and compound **14** (0.05 g, 0.34 mmol). Purification by typical silica gel flash chromatography (CH_2Cl_2/MeOH, from 100:0 to 95:5) afforded the pure title compound (0.063 g, 71%), as a white solid. $R_t = 1.95$ min; MS (ESI) *m/z*: 261 $[M-H]^+$, 283 $[M-Na]^+$, 299 $[M-K]^+$. HRMS (*m/z*): $[M-H]^+$ calculated 261.1239, found 261.1243. ^{1}H NMR (DMSO-d_6): δ 7.91 (s, 1H), 7.85 (d, 1H, $J = 8.8$ Hz), 7.40–7.23 (m, 5H), 6.52 (dt, 1H, $J = 11.7$, 1.8 Hz), 5.71–5.62 (m, 1H), 4.67–4.50 (m, 1H), 4.08 (td, 2H, $J = 6.8$, 1.8 Hz), 3.37 (t, 1H, $J = 5.3$ Hz), 3.06 (dd, 1H, $J = 5.3$, 2.8 Hz), 2.59 (qd, 2H, $J = 6.8$, 1.8 Hz). ^{13}C NMR (DMSO-d_6): δ 168.14, 155.44, 136.68, 130.58, 128.47, 128.29, 128.11, 126.85, 63.62, 58.30, 42.59, 28.30.

5.1.21. (3-Cyclohexylphenyl)-methyl-N-[(S)-2-oxoazetidin-3-yl]-carbamate (3u)

*5.1.21.1. (3-Cyclohexylphenyl)-methyl-2-pyridyl carbonate (**12u**) and (3-cyclohexylphenyl)-methyl-2-oxopyridine-1-carboxylate (**13u**).* General Procedure (Step 1) was followed using (3-cyclohexylphenyl)methanol (**11u**) (0.19 g, 1.0 mmol). A mixture (1:1.7 ratio) of (3-cyclohexylphenyl)-methyl-2-pyridyl carbonate (**12u**) and (3-cyclohexylphenyl)-methyl-2-oxopyridine-1-carboxylate (**13u**) was not separated and used in the next step without any further purification. $R_t = 2.92$ min; MS (ESI) *m/z*: 312 $[M-H]^+$, 334 $[M-Na]^+$.

*5.1.21.2. (3-Cyclohexylphenyl)-methyl-N-[(S)-2-oxoazetidin-3-yl]-carbamate (**3u**).* General Procedure (Step 2) was followed using the previously described mixture (0.327 g) containing **13u** and compound **14** (0.05 g, 0.46 mmol). Purification by silica gel flash chromatography using a Teledyne ISCO apparatus (cyclohexane/TBME from 50:50 to 0:100) afforded the pure title compound (0.072 g, 70%) as a white solid. $R_t = 2.54$ min; MS (ESI) *m/z*: 303 $[M-H]^+$, 320 $[M-NH_4]^+$, 325 $[M-Na]^+$, 341 $[M-K]^+$. HRMS (*m/z*): $[M-H]^+$ calculated: 303.1709, found 303.1714. ^{1}H NMR (DMSO-d_6): δ 8.04–7.87 (m, 2H), 7.27 (t, 1H, $J = 7.5$ Hz), 7.20 (d, 1H, $J = 1.7$ Hz), 7.16 (td, 2H, $J = 7.5$, 1.7 Hz), 5.00 (s, 2H), 4.66 (ddd, 1H, $J = 8.6$, 5.5, 2.7 Hz), 3.39 (t, 1H, $J = 5.5$ Hz), 3.08 (dd, 1H, $J = 5.5$, 2.7 Hz), 2.46 (dd, 1H, $J = 7.3$, 4.1 Hz), 1.77 (ddd, 4H, $J = 11.6$, 5.1, 2.4 Hz), 1.70 (dd, 1H, $J = 12.9$, 3.6 Hz), 1.46–1.13 (m, 6H). ^{13}C NMR (DMSO-d_6): δ 168.10, 155.38, 147.75, 136.64, 128.35, 126.29, 126.26, 125.44, 65.83, 58.37, 43.73, 42.66, 33.95, 26.34, 25.59.

5.1.22. (2-Cyclohexylphenyl)-methyl-N-[(S)-2-oxoazetidin-3-yl]-carbamate (3v)

*5.1.22.1. (2-Cyclohexylphenyl)-methyl-2-pyridyl carbonate (**12v**) and (2-cyclohexylphenyl)-methyl-2-oxopyridine-1-carboxylate (**13v**).* General Procedure (Step 1) was followed using (2-cyclohexylphenyl)-methanol (**11v**) (0.3 g, 1.58 mmol). A mixture (1:1.7 ratio) of (2-cyclohexylphenyl)-methyl-2-pyridyl carbonate (**12v**) and (2-cyclohexylphenyl)-methyl-2-oxopyridine-1-carboxylate (**13v**) was not separated and used in the next step without any further purification. $R_t = 2.89$ min; MS (ESI) *m/z*: 312 $[M-H]^+$, 334 $[M-Na]^+$.

*5.1.22.2. (2-Cyclohexylphenyl)-methyl-N-[(S)-2-oxoazetidin-3-yl]-carbamate (**3v**).* General Procedure (Step 2) was followed using the previously described mixture (0.39 g) containing **13v** and compound **14** (0.067 g, 0.46 mmol). Purification by typical silica gel column chromatography (isocratic cyclohexane/EtOAc 85:15) afforded the pure title compound (0.062 g, 45%), as a white solid. $R_t = 2.47$ min; MS (ESI) *m/z*: 303 $[M-H]^+$, 320 $[M-Na]^+$. HRMS (*m/z*): $[M-H]^+$ calculated: 303.1709, found 303.1710. ^{1}H NMR (DMSO-d_6): δ 7.94–7.86 (m, 2H), 7.36–7.24 (m, 3H), 7.21–7.10 (m, 1H), 5.09 (s, 2H), 4.70–4.61 (m, 1H), 3.39 (t, 1H, $J = 5.37$ Hz), 3.10–3.03 (m, 1H), 2.75–2.65 (m, 1H), 1.82–1.68 (m, 6H), 1.48–1.21 (m, 6H). ^{13}C NMR (DMSO-d_6): δ 168.07, 155.27, 146.31, 133.38, 129.43, 128.48, 125.92, 125.52, 63.75, 58.40, 42.68, 38.95, 33.73, 26.53, 25.67.

5.1.23. (4-Cyclohexylphenyl)-methyl-N-[(S)-2-oxoazetidin-3-yl]-carbamate (3w)

*5.1.23.1. (4-Cyclohexylphenyl)-methyl-2-pyridyl carbonate (**12w**) and (4-cyclohexylphenyl)-methyl-2-oxopyridine-1-carboxylate (**13w**).* General Procedure (Step 1) was followed using (4-cyclohexylphenyl)-methanol (**11w**) (0.233 g, 1.23 mmol). A mixture (1:1.7 ratio) of (4-cyclohexylphenyl)-methyl-2-pyridyl carbonate (**12w**) and (4-cyclohexylphenyl)-methyl-2-oxopyridine-1-carboxylate (**13w**) was not separated and used in the next step without any further purification. $R_t = 1.73$ min; MS (ESI) *m/z*: 268 $[M-H]^+$.

*5.1.23.2. (4-Cyclohexylphenyl)-methyl-N-[(S)-2-oxoazetidin-3-yl]-carbamate (**3w**).* General Procedure (Step 2) was followed using the previously described mixture (0.327 g) containing **13w** and compound **14** (0.043 g, 0.29 mmol). Purification by typical silica gel column chromatography (isocratic cyclohexane/EtOAc 85:15) afforded the pure title compound (0.035 g, 38%), as a white solid. $R_t = 2.57$ min; MS (ESI) *m/z*: 303 $[M-H]^+$, 320 $[M-NH_4]^+$. HRMS (*m/z*): $[M-H]^+$ calculated: 303.1709, found 303.1708. ^{1}H NMR (DMSO-d_6): δ 7.95–7.88 (m, 2H), 7.29–7.17 (m, 4H), 4.98 (s, 2H), 4.65 (ddd, 1H, $J = 8.4$, 5.3, 2.7 Hz), 3.39 (t, 1H, $J = 5.3$ Hz), 3.07 (dd, 1H, $J = 5.3$, 2.7 Hz), 2.47–2.44 (m, 1H), 1.83–1.72 (m, 4H), 1.72–1.64 (m, 1H), 1.47–1.27 (m, 4H), 1.27–1.13 (m, 1H). ^{13}C NMR (DMSO-d_6): δ 168.06, 155.35, 147.36, 134.17, 128.08, 126.62, 65.56, 58.34, 43.50, 42.64, 33.91, 26.31, 25.55.

5.1.24. (4-Cyclopentylphenyl)-methyl-N-[(S)-2-oxoazetidin-3-yl]-carbamate (3x)

*5.1.24.1. (4-Cyclopentylphenyl)-methyl-2-pyridyl carbonate (**12x**) and (4-cyclopentylphenyl)-methyl-2-oxopyridine-1-carboxylate (**13x**).* General Procedure (Step 1) was followed using (4-cyclopentylphenyl)-methanol (**11x**) (0.225 g, 1.28 mmol). A mixture (1:2 ratio) of (4-cyclopentylphenyl)-methyl-2-pyridyl carbonate (**12x**) and (4-cyclopentylphenyl)-methyl-2-oxopyridine-1-carboxylate (**13x**) was not separated and used in the next step without any further purification. $R_t = 2.79$ min; MS (ESI) *m/z*: 298 $[M-H]^+$.

*5.1.24.2. (4-Cyclopentylphenyl)-methyl-N-[(S)-2-oxoazetidin-3-yl]-carbamate (**3x**).* General Procedure (Step 2) was followed using the previous described mixture (0.33 g) containing **13x** and compound **14** (0.05 g, 0.34 mmol). Purification by typical silica gel flash chromatography (CH_2Cl_2/MeOH from 100:0 to 95:5) afforded the pure title compound (0.034 g, 35%) as a white solid. $R_t = 2.79$ min; MS (ESI) *m/z*: 298 $[M-H]^+$. HRMS (*m/z*): $[M-H]^+$ calculated: 289.1552, found 289.1560. ^{1}H NMR (DMSO-d_6): δ 7.92 (d, 2H, $J = 9.0$ Hz), 7.32–7.17 (m, 4H), 4.99 (s, 2H), 4.66 (ddd, 1H, $J = 8.4$, 5.4, 2.7 Hz), 3.39 (t, 1H, $J = 5.4$ Hz), 3.08 (dd, 1H, $J = 5.2$, 2.8 Hz), 2.97 (tt, 1H, $J = 9.7$, 7.4 Hz), 2.07–1.94 (m, 2H), 1.76 (tdd, 2H, $J = 9.1$, 6.9, 3.9 Hz), 1.71–1.58 (m, 2H), 1.58–1.44 (m, 2H). ^{13}C NMR (DMSO-d_6): δ 168.10, 155.38, 145.79, 134.12, 128.05, 126.93, 65.57, 58.36, 45.01, 42.66, 34.21, 25.02.

5.1.25. p-Tolylmethyl-N-[(S)-2-oxoazetidin-3-yl]-carbamate (3y)

*5.1.25.1. p-Tolylmethyl-2-pyridyl carbonate (**12y**) and p-tolylmethyl-2-oxopyridine-1-carboxylate (**13y**).* General Procedure (Step 1) was followed using commercially available *p*-tolylmethanol (**11y**) (0.21 g, 1.7 mmol). A mixture (1:1.9 ratio) of *p*-tolylmethyl 2-oxopyridine-1-carboxylate (**12y**) and *p*-tolylmethyl 2-pyridyl carbonate (**13y**) was not separated and used in the next step without any further purification. R_t = 2.15 min. MS (ESI) *m/z*: 244 $[M-H]^+$, 266 $[M-Na]^+$, 282 $[M-K]^+$.

*5.1.25.2. p-Tolylmethyl-N-[(S)-2-oxoazetidin-3-yl]-carbamate (**3y**).* General Procedure (Step 2) was followed using the previously described mixture (0.34 g) containing **13y** and compound **14** (0.05 g, 0.34 mmol). Purification by typical silica gel flash chromatography (CH_2Cl_2/MeOH from 100:0 to 95:5) afforded the pure title compound (0.04 g, 50%), as white solid. R_t = 1.75 min; MS (ESI) *m/z*: 235 $[M-H]^+$, 257 $[M-Na]^+$, 273 $[M-K]^+$. HRMS (*m/z*): $[M-H]^+$ calculated: 235.1083, found 235.108. ^{1}H NMR (DMSO-d_6): δ 7.97–7.88 (m, 2H), 7.24 (d, 2H, J = 7.9 Hz), 7.17 (d, 2H, J = 7.9 Hz), 4.98 (s, 2H), 4.68–4.61 (m, 1H), 3.39 (t, 1H, J = 5.4 Hz), 3.09–3.05 (m, 1H), 2.29 (s, 3H). ^{13}C NMR (DMSO-d_6): δ 168.08, 155.35, 137.19, 133.75, 128.90, 128.04, 65.56, 58.36, 42.64, 20.74.

5.1.26. (4-Ethylphenyl)-methyl-N-[(S)-2-oxoazetidin-3-yl]-carbamate (3z)

*5.1.26.1. (4-Ethylphenyl)-methyl-2-pyridyl carbonate (**12z**) and (4-ethylphenyl)-methyl-2-oxopyridine-1-carboxylate (**13z**).* General Procedure (Step 1) was followed using commercially available 4-ethyl-benzyl alcohol (**11z**) (0.23 mL, 1.7 mmol). A mixture (1:2 ratio) of (4-ethylphenyl)-methyl-2-oxopyridine-1-carboxylate (**12z**) and (4-ethylphenyl)-methyl-2-pyridyl carbonate (**13z**) was not separated and used in the next step without any further purification. R_t = 2.36 min. MS (ESI) *m/z*: 258 $[M-H]^+$, 280 $[M-Na]^+$, 296 $[M-K]^+$.

*5.1.26.2. (4-Ethylphenyl)-methyl-N-[(S)-2-oxoazetidin-3-yl]-carbamate (**3z**).* General Procedure (Step 2) was followed using the previous described mixture (0.33 g) containing **13z** and compound **14** (0.05 g, 0.34 mmol). Purification by typical silica gel flash chromatography (CH_2Cl_2/MeOH from 100:0 to 95:5) afforded the pure title compound (0.054 g, 64%), as white solid. R_t = 1.96 min; MS (ESI) *m/z*: 249 $[M-H]^+$, 271 $[M-Na]^+$, 287 $[M-K]^+$. HRMS (*m/z*): $[M-H]^+$ calculated: 249.1239, found 249.1240.^{1}H NMR (DMSO-d_6): δ 7.97–7.88 (m, 2H), 7.26 (d, 2H, J = 7.9 Hz), 7.20 (d, 2H, J = 7.9 Hz), 4.99 (s, 2H), 4.69–4.61 (m, 1H), 3.39 (t, 1H, J = 5.4 Hz), 3.10–3.04 (m, 1H), 2.59 (q, 2H, J = 7.7 Hz), 1.17 (t, 3H, J = 7.7 Hz). ^{13}C NMR (DMSO-d_6): δ 168.08, 155.37, 143.56, 134.02, 128.11, 127.73, 65.57, 58.35, 42.65, 27.88, 15.62.

5.1.27. (4-Propylphenyl)-methyl-N-[(S)-2-oxoazetidin-3-yl]-carbamate (3aa)

*5.1.27.1. (4-Propylphenyl)-methyl-2-pyridyl carbonate (**12aa**) and (4-propylphenyl)-methyl-2-oxopyridine-1-carboxylate (**13aa**).* General Procedure (Step 1) was followed using (4-propylphenyl)-methanol (**11aa**) (0.2 g, 1.33 mmol). A mixture (1:1.6 ratio) of (4-propylphenyl)-methyl-2-oxopyridine-1-carboxylate (**12aa**) and (4-propylphenyl)-methyl-2-pyridyl carbonate (**13aa**) was not separated and used in the next step without any further purification. R_t = 2.57 min. MS (ESI) *m/z*: 272 $[M-H]^+$, 294 $[M-Na]^+$, 310 $[M-K]^+$.

*5.1.27.2. (4-Propylphenyl)-methyl-N-[(S)-2-oxoazetidin-3-yl]-carbamate (**3aa**).* General Procedure (Step 2) was followed using the previously described mixture (0.24 g) containing **13aa** and compound **14** (0.05 g, 0.34 mmol). Purification by typical silica gel flash chromatography (CH_2Cl_2/MeOH from 100:0 to 95:5) afforded the pure title compound (0.047 g, 53%), as white solid. R_t = 2.17 min; MS (ESI) *m/z*: 263 $[M-H]^+$, 285 $[M-Na]^+$, 301 $[M-K]^+$. HRMS (*m/z*): $[M-H]^+$ calculated 263.1396, found 263.1392. ^{1}H NMR (DMSO-d_6): δ 7.96–7.89 (m, 2H), 7.26 (d, 2H, J = 7.7 Hz), 7.18 (d, 2H, J = 7.7 Hz), 4.99 (s, 2H), 4.69–4.62 (m, 1H), 3.39 (t, 1H, J = 5.3 Hz), 3.09–3.05 (m, 1H), 2.54 (t, 2H, J = 7.5 Hz), 1.57 (m, 2H), 0.88 (t, 3H, J = 7.5 Hz). ^{13}C NMR (DMSO-d_6): δ 168.08, 155.37, 141.91, 134.03, 128.31, 128.02, 65.57, 58.35, 42.64, 36.93, 24.04, 13.59.

5.1.28. (4-Butylphenyl)-methyl-N-[(S)-2-oxoazetidin-3-yl]-carbamate (3ab)

*5.1.28.1. (4-Butylphenyl)-methyl-2-pyridyl carbonate (**12ab**) and (4-butylphenyl)-methyl-2-oxopyridine-1-carboxylate (**13ab**).* General Procedure (Step 1) was followed using commercially available 4-butylbenzyl alcohol (**11ab**) (0.208 mL, 1.22 mmol). A mixture (1:1.65 ratio) of (4-butylphenyl)-methyl-2-pyridyl carbonate (**12ab**) and (4-butylphenyl)-methyl-2-oxopyridine-1-carboxylate (**13ab**) was not separated and used in the next step without any further purification. R_t = 2.79 min; MS (ESI) *m/z*: 286 $[M-H]^+$.

*5.1.28.2. (4-Cyclohexylphenyl)-methyl-N-[(S)-2-oxoazetidin-3-yl]-carbamate (**3ab**).* General Procedure (Step 2) was followed using the previously described mixture (0.28 g) containing **13ab** and compound **14** (0.056 g, 0.38 mmol). Purification by typical silica gel column chromatography (cyclohexane/EtOAc 66:34) afforded the pure title compound (0.07 g, 66%), as a white solid. R_t = 2.39 min; MS (ESI) *m/z*: 277 $[M-H]^+$, 294 $[M-NH_4]^+$. HRMS (*m/z*): $[M-H]^+$ calculated: 277.1552, found 277.1550. ^{1}H NMR (DMSO-d_6): δ 7.98–7.90 (m, 2H), 7.32–1.16 (m, 4H), 5.01 (s, 2H), 4.72–4.61 (m, 1H), 3.40 (t, 1H, J = 5.5 Hz), 3.12–3.04 (m, 1H), 2.58 (t, 2H, J = 7.6 Hz), 1.55 (quint, 2H, J = 7.6 Hz), 1.37–1.23 (m, 2H), 0.90 (t, 3H, J = 7.3 Hz). ^{13}C NMR (DMSO-d_6): δ 168.10, 128.36, 128.22, 65.63, 58.31, 42.58, 34.28, 32.83, 22.36, 13.97.

5.1.29. (4-tert-Butylphenyl)-methyl-N-[(S)-2-oxoazetidin-3-yl]-carbamate (3ac)

*5.1.29.1. (4-tert-Butylphenyl)-methyl-2-pyridyl carbonate (**12ac**) and (4-tert-butylphenyl)-methyl-2-oxopyridine-1-carboxylate (**13ac**).* General Procedure (Step 1) was followed using commercially available 4-*tert*-butylbenzyl alcohol (**11ac**) (0.22 mL, 1.19 mmol). A mixture (2:1 ratio) of (4-*tert*-butylphenyl)-methyl-2-pyridyl carbonate (**12ac**) and (4-*tert*-butylphenyl)-methyl-2-oxopyridine-1-carboxylate (**13ac**) was not separated and used in the next step without any further purification. R_t = 2.65 min; MS (ESI) *m/z*: 286 $[M-H]^+$.

*5.1.29.2. (4-tert-Butylphenyl)-methyl-N-[(S)-2-oxoazetidin-3-yl]-carbamate (**3ac**).* General Procedure (Step 2) was followed using the previous described mixture (0.34 g) containing **13ac** and compound **14** (0.05 g, 0.34 mmol). Purification by typical silica gel flash chromatography (cyclohexane/TBME from 50:50 to 0:100) afforded the pure title compound (0.051 g, 54%) as a white solid. R_t = 2.3 min; MS (ESI) *m/z*: 394 $[M-NH_4]^+$. HRMS (*m/z*): $[M-H]^+$ calculated: 277.1552, found 277.1547. ^{1}H NMR (DMSO-d_6): δ 7.95 (s, 1H), 7.91 (s, 1H), 7.39 (d, 2H, J = 8.3 Hz), 7.28 (d, 2H, J = 8.3 Hz), 5.00 (s, 2H), 4.66 (ddd, 1H, J = 8.4, 5.4, 2.7 Hz), 3.40 (t, 1H, J = 5.4 Hz), 3.08 (dd, 1H, J = 5.3, 2.8 Hz), 1.28 (s, 9H). ^{13}C NMR (DMSO-d_6): δ 168.09, 155.37, 150.43, 133.81, 127.83, 125.10, 65.47, 58.36, 42.66, 34.26, 31.09.

5.1.30. Tetralin-6-yl-methyl-N-[(S)-2-oxoazetidin-3-yl]-carbamate (3ad)

*5.1.30.1. Tetralin-6-ylmethyl-2-pyridyl-carbonate (**12ad**) and tetralin-6-yl-methyl-2-oxopyridine-1-carboxylate (**13ad**).* General Procedure (Step 1) was followed using tetralin-6-yl-methanol (**11ad**) (0.2 g, 1.23 mmol). A mixture (2:1 ratio) of tetralin-6-ylmethyl-2-pyridyl-carbonate (**12ad**) and tetralin-6-yl-methyl-2-oxopyridine-1-carboxylate (**13ad**) was not separated and used in the next step without any further purification. R_t = 2.57 min; MS (ESI) *m/z*: 284 $[M-H]^+$.

*5.1.30.2. Tetralin-6-yl-methyl-N-[(S)-2-oxoazetidin-3-yl]-carbamate (**3ad**).* General Procedure (Step 2) was followed using the previously described mixture (0.35 g) containing **13ad** and compound **14** (0.05 g, 0.34 mmol). Purification by typical silica gel flash chromatography (cyclohexane/EtOAc from 70:30 to 30:70) afforded the pure title compound (0.056 g, 56%) as a white solid. R_t = 2.15 min; MS (ESI) *m/z*: 292 $[M-NH_4]^+$. HRMS (*m/z*): $[M-H]^+$ calculated: 275.1396, found 275.1402. ^{1}H NMR (DMSO-d_6): δ 7.95–7.85 (m, 2H), 7.03 (s, 3H), 4.94 (s, 2H), 4.65 (ddd, 1H, *J* = 8.5, 6.3, 2.7 Hz), 3.39 (t, 1H, *J* = 5.4 Hz), 3.07 (dd, 1H, *J* = 5.3, 2.8 Hz), 2.76–2.64 (m, 4H), 1.79–1.66 (m, 4H). ^{13}C NMR (DMSO-d_6): δ 168.12, 155.38, 136.54, 136.38, 133.71, 128.90, 128.62, 125.24, 65.66, 58.36, 42.66, 28.74, 28.53, 22.69, 22.67.

5.1.31. Indan-5-ylmethyl-N-[(S)-2-oxoazetidin-3-yl]-carbamate (3ae)

*5.1.31.1. Indan-5-ylmethyl-2-pyridyl carbonate (**12ae**) and indan-5-ylmethyl 2-oxo-pyridine-1-carboxylate (**13ae**).* General Procedure (Step 1) was followed using commercially available indan-5-ylmethanol (**11ae**) (0.2 g, 1.35 mmol). A mixture (2:1 ratio) of indan-5-ylmethyl-2-pyridyl carbonate (**12ae**) and indan-5-ylmethyl 2-oxo-pyridine-1-carboxylate (**13ae**) was not separated and used in the next step without any further purification. R_t = 2.67 min; MS (ESI) *m/z*: 270 $[M-H]^+$.

*5.1.31.2. Indan-5-ylmethyl-N-[(S)-2-oxoazetidin-3-yl]-carbamate (**3ae**).* General Procedure (Step 2) was followed using the previously described mixture (0.35 g) containing **13ae** and compound **14** (0.05 g, 0.34 mmol). Purification by typical silica gel flash chromatography (cyclohexane/TBME from 100:0 to 0:100) afforded the pure title compound (0.02 g, 22%) as a white solid. R_t = 1.98 min; MS (ESI) *m/z*: 278 $[M-NH_4]^+$. HRMS (*m/z*): $[M-H]^+$ calculated: 261.1239, found 261.1238. ^{1}H NMR (DMSO-d_6): δ 8.03–7.76 (m, 2H), 7.22–7.16 (m, 2H), 7.09 (d, 1H, *J* = 7.7 Hz), 4.98 (s, 2H), 4.65 (m, 1H), 3.38 (t, 1H, *J* = 8.0 Hz), 3.08–3.06 (m, 1H), 2.87–2.80 (m, 4H), 2.00 (q, 1H, *J* = 8.0 Hz). ^{13}C NMR (DMSO-d_6): δ 168.09, 155.36, 143.87, 143.49, 134.51, 126.08, 124.11, 124.05, 65.85, 58.34, 42.64, 32.15, 32.02, 25.06.

5.1.32. [(1R)- and (1S)-1-(4-cyclohexylphenyl)-ethyl]-N-[(S)-2-oxoazetidin-3-yl]-carbamate (3af)

*5.1.32.1. (R/S)-1-(4-cyclohexylphenyl)-ethyl-2-pyridyl carbonate (**12af**) and (R/S)-1-(4-cyclohexylphenyl)-ethyl-2-oxopyridine-1-carboxylate (**13af**).* General Procedure (Step 1) was followed using (*R/S*)-1-(4-cyclohexylphenyl)-ethanol (**11af**) (0.2 g, 1.0 mmol). A mixture (1:1.8 ratio) of (*R*/S)-1-(4-cyclohexylphenyl)-ethyl-2-pyridyl carbonate (**12af**) and (*R*/S)-1-(4-cyclohexylphenyl)-ethyl-2-oxopyridine-1-carboxylate (**13af**) was not separated and used in the next step without any further purification.

*5.1.32.2. (1R)-1-(4-cyclohexylphenyl)-ethyl-N-[(S)-2-oxoazetidin-3-yl]-carbamate and (1S)-1-(4-cyclohexylphenyl)-ethyl-N-[(S)-2-oxoazetidin-3-yl]-carbamate (**3af**).* General Procedure (Step 2) was followed using the previous described mixture (0.35 g) containing **13af** and compound **14** (0.05 g, 0.34 mmol). Purification by typical silica gel flash chromatography (CH_2Cl_2/MeOH from 100:0 to 95:5) afforded the pure title compound (0.057 g, 53%), as white solid. R_t = 2.65 min; MS (ESI) *m/z*: 317 $[M-H]^+$, 339 $[M-Na]^+$, 355 $[M-K]^+$. HRMS (*m/z*): $[M-H]^+$ calculated: 317.1865, found 317.1877. ^{1}H NMR (DMSO-d_6): δ 7.98–7.86 (m, 2H), 7.27–7.23 (m, 2H), 7.22–7.17 (m, 2H), 5.69–5.61 (m, 1H), 4.66–4.58 (m, 1H), 3.41–3.33 (m, 1H), 3.07–3.01 (m, 1H), 1.84–1.65 (m, 5H), 1.47–1.15 (m, 9H). ^{13}C NMR (DMSO-d_6): δ 168.16, 154.83, 146.90, 139.61, 126.58, 125.76, 71.62, 58.27, 43.47, 42.71, 33.92, 26.33, 25.57, 22.29.

5.1.33. (4-Tetrahydropyran-4-yl-phenyl)-methyl-N-[(S)-2-oxoazetidin-3-yl]-carbamate (3ag)

*5.1.33.1. (4-Tetrahydropyran-4-yl-phenyl)-methyl-2-pyridyl-carbonate (**12ag**) and (4-tetrahydropyran-4-yl-phenyl)-methyl-2-oxopyridine-1-carboxylate (**13ag**).* General Procedure (Step 1) was followed using (4-tetrahydropyran-4-yl-phenyl)-methanol (**11ag**) (0.16 g, 0.83 mmol). A mixture (1:2 ratio) of 2-pyridyl-(4-tetrahydropyran-4-yl-phenyl)-methyl carbonate (**12ag**) and (4-tetrahydropyran-4-yl-phenyl)-methyl-2-oxopyridine-1-carboxylate (**13ag**) was not separated and used in the next step without any further purification. R_t = 2.05 min; MS (ESI) *m/z*: 314 $[M-H]^+$.

*5.1.33.2. (4-Tetrahydropyran-4-yl-phenyl)-methyl-N-[(S)-2-oxoazetidin-3-yl]-carbamate (**3ag**).* General Procedure (Step 2) was followed using the previously described mixture (0.27 g) containing **13ag** and compound **14** (0.04 g, 0.37 mmol). Purification by typical silica gel flash chromatography (cyclohexane/TBME from 30:70 to 0:100) afforded the pure title compound (0.049 g, 59%) as a white solid. R_t = 1.38 min; MS (ESI) *m/z*: 305 $[M-H]^+$, 322 $[M-NH_4]^+$. HRMS (*m/z*): $[M-H]^+$ calculated: 305.1501, found 305.1503. ^{1}H NMR (DMSO-d_6): δ 7.93 (s, 1H), 7.91 (s, 1H), 7.29 (d, 2H, *J* = 8.2 Hz), 7.25 (d, 2H, *J* = 8.2 Hz), 5.00 (s, 2H), 4.65 (m, 1H), 3.94 (dt, 2H, *J* = 11.3, 3.1 Hz), 3.48–3.36 (m, 3H), 3.07 (dd, 1H, *J* = 5.2, 2.8 Hz), 2.84–2.69 (m, 1H), 1.75–1.57 (m, 4H). ^{13}C NMR (DMSO-d_6): δ 168.09, 155.36, 145.74, 134.57, 128.17, 126.68, 67.34, 65.51, 58.35, 42.66, 33.50.

5.1.34. [4-(Cyclohexoxy)-phenyl]-methyl-N-[(S)-2-oxoazetidin-3-yl]-carbamate (3ah)

*5.1.34.1. [4-(Cyclohexoxy)-phenyl]-methyl-2-pyridyl carbonate (**12ah**) and [4-(cyclohexoxy)-phenyl]-methyl-2-oxopyridine-1-carboxylate (**13ah**).* General Procedure (Step 1) was followed using [4-(cyclohexoxy)-phenyl]-methanol (**11ah**) (0.55 g, 2.67 mmol). A mixture (1.7:1 ratio) of [4-(cyclohexoxy)-phenyl]-methyl-2-pyridyl carbonate (**12ah**) and [4-(cyclohexoxy)-phenyl]-methyl-2-oxopyridine-1-carboxylate (**13ah**) was not separated and used in the next step without any further purification. R_t = 2.79 min; MS (ESI) *m/z*: 366 $[M-K]^+$.

*5.1.34.2. [4-(Cyclohexoxy)-phenyl]-methyl-N-[(S)-2-oxoazetidin-3-yl]-carbamate (**3ah**).* General Procedure (Step 2) was followed using the previously described mixture (0.32 g) containing **13ah** and compound **14** (0.052 g, 0.36 mmol). Purification by typical silica gel column chromatography (cyclohexane/EtOAc 85:15) afforded the pure title compound (0.065 g, 57%), as a white solid. R_t = 2.39 min; MS (ESI) *m/z*: 336 $[M-NH_4]^+$, 357 $[M-K]^+$. HRMS (*m/z*): 341.1482 $[M-Na]^+$ calculated: 341.1477, found 341.1482. ^{1}H NMR (DMSO-d_6): δ 7.88–7.79 (m, 2H), 7.26 (d, 2H, *J* = 8.3 Hz), 6.91 (d, 2H, *J* = 8.3 Hz), 4.96 (s, 2H), 4.70–4.61 (m, 1H), 4.38–4.27 (m, 1H), 3.44–3.36 (m, 1H), 3.12–3.05 (m, 1H), 1.96–1.86 (m, 2H), 1.77–1.66 (m, 2H), 1.59–1.23 (m, 6H). ^{13}C NMR (DMSO-d_6): δ 168.10, 129.79, 115.51, 74.12, 64.09, 58.26, 42.70, 31.35, 25.24, 22.99.

5.1.35. (4-Propoxyphenyl)-methyl-N-[(S)-2-oxoazetidin-3-yl]-carbamate (3ai)

*5.1.35.1. (4-Propoxyphenyl)-methyl-2-pyridyl carbonate (**12ai**) and (4-propoxyphenyl)-methyl-2-oxopyridine-1-carboxylate (**13ai**).* General Procedure (Step 1) was followed using (4-propoxyphenyl)-methanol (**11ai**) (0.23 g, 1.4 mmol). A mixture (1:2.4 ratio) of (4-propoxyphenyl)-methyl-2-pyridyl carbonate (**12ai**) and (4-propoxyphenyl)-methyl-2-oxopyridine-1-carboxylate (**13ai**) was not separated and used in the next step without any further purification. R_t = 2.41 min; MS (ESI) *m/z*: 286 $[M-H]^-$.

*5.1.35.2. (4-Propoxyphenyl)methyl-N-[(S)-2-oxoazetidin-3-yl]-carbamate (**3ai**).* General Procedure (Step 2) was followed using the previously described mixture (0.26 g) containing **13ai** and compound **14** (0.025 g, 0.17 mmol). Purification by typical silica gel flash chromatography (cyclohexane/EtOAc 50:50) afforded the pure title compound (0.038 g, 78%) as a white solid. R_t = 2.02 min; MS (ESI) *m/z*: 296 $[M-NH_4]^+$, 301 $[M-Na]^+$, 317 $[M-K]^+$. HRMS (*m/z*): $[M-H]^+$ calculated: 279.1345, found 279.1353. ^{1}H NMR (DMSO-d_6): δ 8.13–7.78 (m, 2H), 7.35–7.21 (m, 2H), 6.98–6.86 (m, 2H), 4.95 (s, 2H), 4.72–4.57 (m, 1H), 3.91 (t, 2H, *J* = 7.0 Hz), 3.38 (t, 1H, *J* = 5.3 Hz), 3.07 (dd, 1H, *J* = 2.7, 5.3 Hz), 1.72 (m, 2H), 0.97 (t, 3H, *J* = 7.4 Hz). ^{13}C NMR (DMSO-d_6): δ 168.11, 158.49, 155.40, 128.51, 114.26, 68.92, 65.49, 58.34, 42.63, 21.98, 10.36.

5.1.36. 1,3-Benzodioxol-5-yl-methyl-N-[(S)-2-oxoazetidin-3-yl]-carbamate (3aj)

*5.1.36.1. 1,3-Benzodioxol-5-yl-methyl-2-pyridyl carbonate (**12aj**) and 1,3-benzodioxol-5-yl-methyl-2-oxopyridine-1-carboxylate (**13aj**).* General Procedure (Step 1) was followed using commercially available 1,3-benzodioxol-5-yl-methanol (**11aj**) (0.30 g, 1.97 mmol). A mixture (1.58:1 ratio) of 1,3-benzodioxol-5-ylmethyl-2-pyridyl carbonate (**13aj**) and 1,3-benzodioxol-5-ylmethyl-2-oxopyridine-1-carboxylate (**12aj**) was not separated and used in the next step without any further purification. R_t = 1.91 min. MS (ESI) *m/z*: 296 $[M-Na]^+$, 312 $[M-K]^+$.

*5.1.36.2. 1,3-Benzodioxol-5-yl-methyl-N-[(S)-2-oxoazetidin-3-yl]-carbamate (**3aj**).* General Procedure (Step 2) was followed using the previously described mixture (0.28 g) containing **13aj** and compound **14** (0.060 g, 0.41 mmol). Purification by silica gel flash chromatography using a Teledyne ISCO apparatus (cyclohexane/TBME from 100:0 to 0:100) and trituration with heptane afforded the pure title compound (0.053 g, 49%), as a white solid. R_t = 1.51 min; MS (ESI) *m/z*: 265 $[M-H]^+$, 282 $[M-NH_4]^+$, 287 $[M-Na]^+$, 303 $[M-K]^+$. HRMS (*m/z*): $[M-H]^+$ calculated 265.0824, found 265.0823. ^{1}H NMR (DMSO-d_6): δ 8.00–7.85 (m, 2H), 6.95–6.82 (m, 3H), 6.01 (s, 2H), 4.92 (s, 2H), 4.65 (ddd, 1H, *J* = 8.3, 5.4, 2.7 Hz), 3.39 (t, 1H, *J* = 5.4 Hz), 3.07 (dd, 1H, *J* = 5.4, 2.7 Hz). ^{13}C NMR (DMSO-d_6): δ 168.07, 154.11, 148.15, 147.23, 130.44, 121.95, 108.08, 100.99, 99.51, 65.61, 58.33, 42.63.

5.1.37. (2,2-Difluoro-1,3-benzodioxol-5-yl)-methyl-N-[(S)-2-oxoazetidin-3-yl]-carbamate (3ak)

*5.1.37.1. (2,2-Difluoro-1,3-benzodioxol-5-yl)-methyl-2-pyridyl carbonate (**12ak**) and (2,2-difluoro-1,3-benzodioxol-5-yl)-methyl-2-oxopyridine-1-carboxylate (**13ak**).* General Procedure (Step 1) was followed using (2,2-difluoro-1,3-benzodioxol-5-yl)-methanol (**11ak**) (0.30 g, 1.59 mmol). A mixture (1.8:1 ratio) of (2,2-difluoro-1,3-benzodioxol-5-yl)-methyl-2-pyridyl carbonate (**12ak**) and (2,2-difluoro-1,3-benzodioxol-5-yl)-methyl-2-oxopyridine-1-carboxylate (**13ak**) was not separated and used in the next step without any further purification. R_t = 2.34 min. MS (ESI) *m/z*: 310 $[M-H]^+$, 332 $[M-Na]^+$, 348 $[M-K]^+$.

*5.1.37.2. (2,2-Difluoro-1,3-benzodioxol-5-yl)-methyl-N-[(S)-2-oxoazetidin-3-yl]-carbamate (**3ak**).* General Procedure (Step 2) was followed using the previously described mixture (0.34 g) containing **13ak** and compound **14** (0.060 g, 0.41 mmol). Purification by silica gel flash chromatography using a Teledyne ISCO apparatus (cyclohexane/TBME from 100:0 to 0:100) afforded the pure title compound (0.053 g, 43%), as a white solid. R_t = 1.97 min; MS (ESI) *m/z*: 301 $[M-H]^+$, 318 $[M-NH_4]^+$, 323 $[M-Na]^+$, 339 $[M-K]^+$. HRMS (*m/z*): $[M-H]^+$ calculated: 301.0636, found 301.0639. ^{1}H NMR (DMSO-d_6): δ 7.99 (d, 1H, *J* = 8.5 Hz), 7.94 (bs, 1H), 7.45–7.33 (m, 2H), 7.22 (dd, 1H, *J* = 8.3, 1.4 Hz), 5.04 (s, 2H), 4.66 (ddd, 1H, *J* = 8.5, 5.4, 2.5 Hz), 3.39 (t, 1H, *J* = 5.4 Hz), 3.08 (dd, 1H, *J* = 5.4, 2.5 Hz). ^{13}C NMR (DMSO-d_6): δ 168.2, 155.3, 142.8, 142.5, 133.8, 131.3 (t, J_{CF} = 253 Hz), 124.5, 110.1, 110.1, 65.2, 58.4, 42.8.

5.1.38. [4-(3-Pyridyl)-phenyl]-methyl-N-[(S)-2-oxoazetidin-3-yl]-carbamate (3al)

*5.1.38.1. [4-(3-Pyridyl)-phenyl]-methyl-2-pyridyl carbonate (**12al**) and [4-(3-pyridyl)-phenyl]-methyl-2-oxopyridine-1-carboxylate (**13al**).* General Procedure (Step 1) was followed using commercially available [4-(3-pyridyl)-phenyl]-methanol (**11al**) (0.25 g, 1.35 mmol). A mixture (1:1.7 ratio) of [4-(3-pyridyl)-phenyl]-methyl-2-pyridyl carbonate (**12al**) and [4-(3-pyridyl)-phenyl]-methyl-2-oxopyridine-1-carboxylate (**13al**) was not separated and used in the next step without any further purification. R_t = 1.86 min. MS (ESI) *m/z:* 307 $[M-H]^+$, 329 $[M-Na]^+$, 345 $[M-K]^+$.

*5.1.38.2. [4-(3-Pyridyl)]-methyl-N-[(S)-2-oxoazetidin-3-yl]-carbamate(**3al**).* General Procedure (Step 2) was followed using the previously described mixture (0.28 g) containing **13al** and compound **14** (0.050 g, 0.34 mmol). Purification by preparative HPLC afforded the pure title compound (0.014 g, 14%), as a white solid. R_t = 1.54 min; MS (ESI) *m/z*: 298 $[M-H]^+$, 320 $[M-Na]^+$, 336 $[M+K]^+$. HRMS (*m/z*): $[M-H]^+$ calculated: 298.1192, found 298.1196. ^{1}H NMR (DMSO-d_6): δ 8.90 (dd, 1H, *J* = 2.4, 0.7 Hz), 8.57 (dd, 1H, *J* = 4.8, 1.6 Hz), 8.08 (ddd, 1H, *J* = 8.0, 2.4, 1.7 Hz), 8.02 (d, 1H, *J* = 8.8 Hz), 7.96 (s, 1H), 7.77–7.71 (m, 2H), 7.52–7.46 (m, 3H), 5.10 (s, 2H), 4.67 (ddd, 1H, *J* = 8.4, 5.5, 2.8 Hz), 3.40 (t, 1H, *J* = 5.5 Hz), 3.10 (dd, 1H, *J* = 5.5, 2.8 Hz). ^{13}C NMR (DMSO-d_6): δ 168.12, 155.38, 148.59, 147.68, 136.85, 136.69, 135.21, 134.15, 128.66, 126.97, 123.92, 65.31, 58.39, 42.71.

5.1.39. (4-Oxazol-4-ylphenyl)-methyl-N-[(S)-2-oxoazetidin-3-yl]-carbamate (3am)

*5.1.39.1. (4-Oxazol-4-ylphenyl)-methyl-2-pyridyl carbonate (**12am**) and (4-oxazol-4-ylphenyl)-methyl-2-oxopyridine-1-carboxylate (**13am**).* General Procedure (Step 1) was followed using (4-oxazol-4-ylphenyl)-methanol (**11am**) (0.18 g, 1.03 mmol). A mixture (2:1 ratio) of (4-oxazol-4-ylphenyl)-methyl-2-pyridyl carbonate (**12am**) and (4-oxazol-4-ylphenyl)-methyl-2-oxopyridine-1-carboxylate (**13am**) was not separated and used in the next step without any further purification. R_t = 2.14 min; MS (ESI) *m/z*: 297 $[M-H]^+$.

*5.1.39.2. (4-Oxazol-4-ylphenyl)-methyl-N-[(S)-2-oxoazetidin-3-yl]-carbamate (**3am**).* General Procedure (Step 2) was followed using the previously described mixture (0.32 g) containing **13am** and compound **14** (0.05 g, 0.34 mmol). The resulting crude was triturated in CH_2Cl_2 (2.0 mL) to afford the pure title compound (0.032 g, 33%) as a white solid. R_t = 1.49 min; MS (ESI) *m/z*: 288 $[M-H]^+$. HRMS (*m/z*): $[M-H]^+$ calculated: 288.0984, found 288.9750. ^{1}H NMR (DMSO-d_6): δ 8.63 (s, 1H), 8.46 (s, 1H), 8.04–7.89 (m, 2H), 7.79 (d, 2H, *J* = 8.1 Hz), 7.42 (d, 2H, *J* = 8.0 Hz), 5.06 (s, 2H), 4.67 (m, 1H), 3.39 (m, 1H), 3.09 (m, 1H). ^{13}C NMR (DMSO-d_6): δ 168.07, 155.34,

152.65, 138.90, 136.50, 135.23, 130.36, 128.39, 125.22, 65.40, 58.38, 42.65.

5.2. Synthesis of [(R)-2-oxoazetidin-3-yl]-ammonium acetate (**15**)

Off-white solid. Experimental procedure and ^{1}H NMR are according to literature [42].

5.2.1. 4-Cyclohexylbutyl-N-[(R)-2-oxoazetidin-3-yl]-carbamate (4b)

Experimental procedure, ^{1}H and ^{13}C NMR are according to literature [8]. HRMS (*m/z*): $[M-H]^+$ calculated 269.1845, found 269.1849.

5.2.2. 4-Cyclohexylbutyl-N-[(2S,3S)-2-methyl-4-oxo-azetidin-3-yl]-carbamate (5b)

*5.2.2.1. 4-Cyclohexylbutyl-2-pyridyl carbonate (**12b**) and 4-cyclohexylbutyl-2-oxopyridine-1-carboxylate (**13b**).* General Procedure (Step 1) was followed using commercially available 4-cyclohexyl-1-butanol (**11b**) (0.89 mL, 5.13 mmol). The mixture (1:1.7 ratio) of 4-cyclohexylbutyl-2-pyridyl carbonate (**12b**) and 4-cyclohexylbutyl-2-oxopyridine-1-carboxylate (**13b**) was used in the next step without any further purification. R_t = 3.00 min. MS (ESI) *m/z*: 278 $[M-H]^+$, 300 $[M-Na]^+$, 316 $[M-K]^+$.

*5.2.2.2. 4-Cyclohexylbutyl-N-[(2S,3S)-2-methyl-4-oxo-azetidin-3-yl]-carbamate (**5b**).* General Procedure (Step 2) was followed using the previously described mixture (0.069 g) containing **13b** and [(2*S*,3*S*)-2-methyl-4-oxo-azetidin-3-yl]-ammonium toluene-4-sulfonate (**16**) (0.07 g, 0.25 mmol). The resulting crude was purified by column chromatography using a Teledyne ISCO apparatus (cyclohexane/EtOAc from 100:0 to 0:100) to give the pure title compound (0.027 g, 40%), as a white solid. R_t = 2.69 min; MS (ESI) *m/z*: 283 $[M-H]^+$, 305 $[M-Na]^+$, 321 $[M-K]^+$. HRMS (*m/z*): $[M-H]^+$ calculated 283.2022, found 283.2021. ^{1}H NMR (DMSO-d_6): δ 8.05 (s, 1H), 7.77 (d, 1H, J = 8.7 Hz), 4.11–4.04 (m, 1H), 4.00–3.88 (m, 2H), 3.56–3.44 (m, 1H), 1.72–1.56 (m, 5H), 1.56–1.46 (m, 2H), 1.36–1.25 (m, 2H), 1.23 (d, 3H, J = 6.1 Hz), 1.22–1.04 (m, 6H), 0.92–0.77 (m, 2H). ^{13}C NMR (DMSO-d_6): δ 166.75, 155.66, 64.24, 64.11, 51.43, 36.94, 36.48, 32.78, 28.86, 26.18, 25.81, 22.59, 19.13.

5.2.3. 4-Cyclohexylbutyl-N-[(2R,3R)-2-methyl-4-oxo-azetidin-3-yl]-carbamate (6b)

General Procedure (Step 2) was followed using the mixture (0.069 g) containing **13b** *(as described for the preparation of compound **5b**)* and [(2*R*,3*R*)-2-methyl-4-oxo-azetidin-3-yl]-ammonium toluene-4-sulfonate (**17**) (0.09 g, 0.33 mmol). The resulting crude was purified by column chromatography using a Teledyne ISCO apparatus (cyclohexane/EtOAc from 100:0 to 0:100) to give the pure title compound (0.06 g, 64%), as a white solid. R_t = 2.69 min; MS (ESI) *m/z*: 283 $[M-H]^+$, 305 $[M-Na]^+$, 321 $[M-K]^+$. HRMS (*m/z*): $[M-H]^+$ calculated 283.2022, found 283.2028. ^{1}H NMR (DMSO-d_6): δ 8.05 (s, 1H), 7.77 (d, 1H, J = 8.7 Hz), 4.11–4.04 (m, 1H), 4.00–3.88 (m, 2H), 3.56–3.44 (m, 1H), 1.72–1.56 (m, 5H), 1.56–1.46 (m, 2H), 1.36–1.25 (m, 2H), 1.23 (d, 3H, J = 6.1 Hz), 1.22–1.04 (m, 6H), 0.92–0.77 (m, 2H). ^{13}C NMR (DMSO-d_6): δ 166.76, 155.66, 64.24, 64.12, 51.44, 36.96, 36.49, 32.79, 28.87, 26.19, 25.82, 22.61, 19.14.

5.2.4. 4-Cyclohexylbutyl-N-[(2R,3S)-2-methyl-4-oxo-azetidin-3-yl]-carbamate (7b)

General Procedure (Step 2) was followed using the mixture (0.075 g) containing **13b** *(as described for the preparation of compound **5b**)* and [(2*R*,3*S*)-2-methyl-4-oxo-azetidin-3-yl]-ammonium toluene-4-sulfonate (**18**) (0.074 g, 0.27 mmol). The resulting crude was purified by typical silica gel column chromatography (cyclohexane/EtOAc 97:3) to afford the pure title compound (0.031 g, 39%), as a white solid. R_t = 2.71 min; MS (ESI) *m/z*: 283 $[M-H]^+$, 305 $[M-Na]^+$, 321 $[M-K]^+$. HRMS (*m/z*): $[M-H]^+$ calculated 283.2022, found 283.2016. ^{1}H NMR (DMSO-d_6): δ 8.10 (s, 1H), 7.85 (d, 1H, J = 9.5 Hz), 4.87–4.65 (m, 1H), 4.01–3.90 (m, 2H), 3.79–3.68 (m, 1H), 1.72–1.56 (m, 5H), 1.56–1.46 (m, 2H), 1.36–1.10 (m, 8H), 1.06 (d, 3H, J = 6.2 Hz), 0.91–0.77 (m, 2H). ^{13}C NMR (DMSO-d_6): δ 168.21, 155.56, 64.15, 60.15, 48.74, 36.96, 36.48, 32.78, 28.90, 26.18, 25.81, 22.57, 15.70.

5.2.5. 4-Cyclohexylbutyl-N-[(2S,3R)-2-methyl-4-oxo-azetidin-3-yl]-carbamate (8b)

General Procedure (Step 2) was followed using the mixture (0.083 g) containing **13b** *(as described for the preparation of compound **5b**)* and [(2*S*,3*R*)-2-methyl-4-oxo-azetidin-3-yl]-ammonium toluene-4-sulfonate (**19**) (0.08 g, 0.30 mmol). The resulting crude was purified by typical silica gel column chromatography (Cy/EtOAc 97:3) to afford the pure title compound (0.031 g, 39%), as a white solid. R_t = 2.71 min; MS (ESI) *m/z*: 283 $[M-H]^+$, 305 $[M-Na]^+$, 321 $[M-K]^+$. HRMS (*m/z*): $[M-H]^+$ calculated 283.2022, found 283.2025. ^{1}H NMR (DMSO-d_6): δ 8.10 (s, 1H), 7.85 (d, 1H, J = 9.5 Hz), 4.87–4.65 (m, 1H), 4.01–3.90 (m, 2H), 3.79–3.68 (m, 1H), 1.72–1.56 (m, 5H), 1.56–1.46 (m, 2H), 1.36–1.10 (m, 8H), 1.06 (d, 3H, J = 6.2 Hz), 0.91–0.77 (m, 2H). ^{13}C NMR (DMSO-d_6): δ 167.23, 156.10, 64.15, 60.15, 48.73, 36.95, 36.48, 32.78, 28.89, 26.17, 25.80, 22.57, 15.70.

5.2.6. 4-Cyclohexylbutyl-N-[(S)-2,2-dimethyl-4-oxo-azetidin-3-yl]-carbamate (9b)

*5.2.6.1. 4-Cyclohexylbutyl-N-[(S)-1-[bis-(trimethylsilyl)-methyl]-2,2-dimethyl-4-oxo-azetidin-3-yl]-carbamate (**22b**).* General Procedure (Step 2) was followed using the mixture (0.105 g) containing **13b** *(as described for the preparation of compound **5b**)* and (*S*)-3-amino-1-[*bis*-(trimethylsilyl)-methyl]-4,4-dimethyl-azetidin-2-one (**20**, see Supporting Information) (0.10 g, 0.37 mmol). The resulting crude was purified by column chromatography using a Teledyne ISCO apparatus (cyclohexane/EtOAc 80:20) to afford a mixture (ratio 1:2.8:1) containing the title compound **22b**, 4-cyclohexylbutyl-2-oxopyridine-1-carboxylate (**12b**) and 4-cyclohexylbutan-1-ol (**11b**) (0.18 g), which was used in the next step without further purification.

*5.2.6.2. 4-Cyclohexylbutyl-N-[(S)-2,2-dimethyl-4-oxo-azetidin-3-yl]-carbamate (**9b**).* To a suspension of the mixture (0.18 g) containing 4-cyclohexylbutyl-*N*-[(3*S*)-1-[*bis*-(trimethylsilyl)-methyl]-2,2-dimethyl-4-oxo-azetidin-3-yl]-carbamate (**22b**) (0.063 g, 0.14 mmol) in MeCN (2.0 mL), cooled to 0 °C, a solution of CAN (0.38 g, 0.70 mmol) in deionized H_2O (0.5 mL) was added. The reaction mixture was stirred at room temperature for 1 h, and then diluted with EtOAc (15 mL) and H_2O (15 mL). The two phases were separated and the aqueous layer was extracted with EtOAc (3 × 15 mL). The combined organic layers were sequentially washed with sat. $NaHCO_3$ solution (10 mL), 40% $NaHSO_3$ solution (5 mL) and with sat. $NaHCO_3$ solution (10 mL). The organic layer was dried over Na_2SO_4 concentrated to dryness to afford a crude mixture (0.175 g) mainly containing 4-cyclohexylbutyl-*N*-[(*S*)-1-formyl-2,2-dimethyl-4-oxo-azetidin-3-yl]-carbamate. This crude was suspended in a 1:1 mixture acetone/sat. $NaHCO_3$ solution (4.0 mL) and stirred at room temperature for 16 h. The suspension was diluted with EtOAc (15 mL) and H_2O (5 mL) was added. After extraction with EtOAc (3 × 15 mL) and the combined organic phases were dried over Na_2SO_4, filtered and concentrated to dryness. The crude product was purified by column chromatography using a Teledyne ISCO apparatus (cyclohexane/EtOAc 50:50) to

afford the pure title compound (0.04 g, 32% over three steps), as white powder. R_t = 1.49 min; MS (ESI) *m/z*: 297 $[M-H]^+$, 314 $[M-NH_4]^+$, 319 $[M-Na]^+$, 335 $[M-K]^+$. HRMS (*m/z*): $[M-H]^+$ calculated 297.2178, found 297.2183. ^{1}H NMR (DMSO-d_6): δ 8.12 (s, 1H), 7.89 (d, 1H, J = 9.2 Hz), 4.34 (d, 1H, J = 9.2 Hz), 4.04–3.89 (m, 2H), 1.71–1.57 (m, 6H), 1.52 (m, 2H), 1.34–1.27 (m, 1H), 1.31 (s, 3H), 1.23–1.16 (m, 5H), 1.14 (s, 3H), 0.93–0.78 (m, 3H). ^{13}C NMR (DMSO-d_6): δ 165.76, 156.24, 65.68, 64.17, 56.36, 36.98, 36.48, 32.80, 28.91, 26.57, 26.20, 25.83, 22.59, 22.38.

5.2.7. 4-Cyclohexylbutyl-N-[(R)-2,2-dimethyl-4-oxo-azetidin-3-yl]-carbamate (10b)

*5.2.7.1. 4-Cyclohexylbutyl-N-[(R)-1-[bis-(trimethylsilyl)-methyl]-2,2-dimethyl-4-oxo-azetidin-3-yl]-carbamate (**23b**).* General Procedure (Step 2) was followed using the mixture (0.11 g) containing **13b** *(as described for the preparation of compound **5b**)* and (*R*)-3-amino-1-[*bis*-(trimethylsilyl)-methyl]-4,4-dimethyl-azetidin-2-one (**21**) (0.12 g, 0.45 mmol). The resulting crude was purified by column chromatography using a Teledyne ISCO apparatus (cyclohexane/EtOAc 80:20) to afford a mixture (ratio 1:2:1) containing the title compound **23b**, 4-cyclohexylbutyl-2-oxopyridine-1-carboxylate (**12b**) and 4-cyclohexylbutan-1-ol (**11b**) (0.25 g), which was used in the next step without further purification.

*5.2.7.2. 4-Cyclohexylbutyl-N-[(R)-2,2-dimethyl-4-oxo-azetidin-3-yl]-carbamate (**10b**).* The procedure reported for the synthesis of compound **9b** was followed using a mixture (0.25 g) containing 4-cyclohexylbutyl-*N*-[(*R*)-1-[*bis*-(trimethylsilyl)-methyl]-2,2-dimethyl-4-oxo-azetidin-3-yl]-carbamate (**23b**) (0.103 g, 0.23 mmol) and CAN (0.38 g, 0.70 mmol). The final crude product was purified by column chromatography using a Teledyne ISCO apparatus (cyclohexane/EtOAc 50:50) to afford the pure title compound (0.04 g, 26% over three steps), as white powder. R_t = 1.49 min; MS (ESI) *m/z*: 297 $[M-H]^+$, 314 $[M-NH_4]^+$, 319 $[M-Na]^+$, 335 $[M-K]^+$. HRMS (*m/z*): $[M-H]^+$ calculated 297.2178, found 297.2184. ^{1}H NMR (DMSO-d_6): δ 8.12 (s, 1H), 7.89 (d, 1H, J = 9.2 Hz), 4.34 (d, 1H, J = 9.2 Hz), 4.04–3.89 (m, 2H), 1.71–1.57 (m, 6H), 1.52 (m, 2H), 1.34–1.27 (m, 1H), 1.31 (s, 3H), 1.23–1.16 (m, 5H), 1.14 (s, 3H), 0.93–0.78 (m, 3H). ^{13}C NMR (DMSO-d_6): δ 165.79, 156.26, 65.66, 64.19, 56.38, 37.00, 36.52, 32.82, 28.93, 26.59, 26.22, 25.85, 22.61, 22.43.

6. Pharmacology

6.1. Preparation of enzyme-enriched lysate

Cells were suspended in 20 mM Tris HCl (pH 7.4) with 0.32 M sucrose, sonicated and centrifuged at 800×*g* for 15 min at 4 °C. Supernatants were then ultracentrifuged at 12,000×*g* for 30 min at 4 °C. Pellets were re-suspended in PBS buffer (pH 7.4) and subjected to three freeze-thaw cycles at −80 °C. The suspension was finally ultracentrifuged at 105,000×*g* for 1 h at 4 °C. For *h-NAAA* and *h-AC* supernatants were collected, while the pellet was re-suspended in PBS to obtain *h-FAAH1* preparation. Protein concentration was measured and samples aliquoted and stored at −80 °C until use.

6.2. Fluorogenic h-NAAA in vitro assay

Hek293 cells stably transfected with the human NAAA coding sequence cloned from a human spleen cDNA library (catalog no. 639124, Clontech, Mountain View, CA, USA) were used as enzyme source. The assay was run in 96-well microplates (Black Opti-Plate™-96 F; PerkinElmer, Massachusetts, USA), in a total reaction volume of 200 μL h-NAAA protein preparation (4.0 μg) was pre-incubated for 10 min with various concentrations of test compounds or vehicle control (5% DMSO) in 100 mM citrate/phosphate buffer (pH 4.5) containing 3.0 mM DTT, 0.1% NP40 0.1%, 0.05% BSA, 150 mM NaCl. *N*-(4-methyl-2-oxo-chromen-7-yl)-hexadecanamide (PAMCA) [67] was used as a substrate (5.0 μM) and the reaction carried for 50 min at 37 °C. Fluorescence was measured with EnVision 2014 Multilabel Reader (PerkinElmer, Massachusetts, USA) using an excitation wavelength of 340 nm and emission 450 nm. IC_{50} values were calculated by non-linear regression analysis of log[concentration]/inhibition curves using GraphPad Prism 5 (GraphPad Software Inc., CA, USA) applying a standard slope curve fitting.

6.3. UPLC-MS h-NAAA in vitro assay

h-NAAA activity in cells was evaluated as previously reported by Armirotti et al. [39].

6.4. UPLC-MS h-AC in vitro assay

h-AC activity in cells was measured with LC-MS based assay as previously described by Pizzirani et al. [68].

6.5. Fluorogenic h-FAAH1 in vitro assay

Hek293 cells stably transfected with the *h-FAAH-1* were used as enzyme source. The fluorescent assay to measure FAAH-1 activity was performed as previously described by De Simone et al. [69].

6.6. In vitro plasma and liver microsomal stability studies in mouse and rat

For a detailed description of in vitro stability studies in mouse and rat, see Supporting Information.

6.7. Aqueous kinetic solubility study

For a detailed description of aqueous kinetic solubility study, see Supporting Information.

Conflict of interest

Authors declare the following financial interest. A.N., A.F., C.P., S.P., A.R., T.B., F.B. and D.P. are inventors in a patent application which protects composition and use of the compounds described in the present study.

Author contributions

A.N., A.F., J.A.O., C.P., S.P. and D.P. synthesised compounds. S.M.B. and G.O. performed plasma and metabolic stability tests, as well as solubility analyses. A.R. and G.T. developed fluorogenic human NAAA in vitro assay. D.P., T.B. and F.B. wrote the manuscript.

Acknowledgements

The authors wish to thank Luisa Mengatto for *h*-NAAA and *h*-AC screenings, Clara Albani for *h*-FAAH-1 screening, Silvia Venzano for compounds handling and Luca Goldoni for NMR support.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2016.01.046.

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