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Azetidinic amino acids: stereocontrolled synthesis and pharmacological characterization as ligands for glutamate receptors and transporters

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A set of ten azetidinic amino acids, that can be envisioned as C-4 alkyl substituted analogues of *trans*-2-carboxyazetidine-3-acetic acid (*t*-CAA) and/or conformationally constrained analogues of (*R*)- or (*S*)-glutamic acid (Glu) have been synthesized in a diastereo- and enantiomerically pure form from β-amino alcohols through a straightforward five step sequence. The key step of this synthesis is an original anionic 4-*exo*-tet ring closure that forms the azetidine ring upon an intramolecular Michael addition. This reaction was proven to be reversible and to lead to a thermodynamic distribution of two diastereoisomers that were easily separated and converted in two steps into azetidinic amino acids. Azetidines **35–44** were characterized in binding studies on native ionotropic Glu receptors and in functional assays at cloned metabotropic receptors mGluR1, 2 and 4, representing group I, II and III mGlu receptors, respectively. Furthermore, azetidine analogues **35**, **36** and **40** were also characterized as potential ligands at the glutamate transporter subtypes EAAT1–3 in the FLIPR® Membrane Potential (FMP) assay. The (2*R*)-azetidines **35**, **37**, **39**, **41** and **43** were inactive in iGlu, mGlu and EAAT assays, whereas a marked change in the pharmacological profile at the iGlu receptors was observed when a methyl group was introduced in the C-4 position, compound **36** *versus t*-CAA. At EAAT1–3, compound **35** was inactive, whereas azetidines **36** and **40** were both identified as inhibitors and showed selectivity for the EAAT2 subtype.

Introduction

Conformationally constrained amino acids (AAs) continue to be a topic of extensive research for both synthetic and medicinal chemists, since such compounds may provide important information about a ligand's conformational binding mode to a receptor or an enzyme, and are basic tools used in the design of novel peptides and peptidomimetics.[1] The synthesis of novel amino acids in which the constraint is brought by a heterocyclic ring which holds the nitrogen atom of the amino acid moiety, has received considerable attention,[2] as illustrated by numerous synthetic approaches towards proline,[3] pipecolic acid,[4] and glutamate analogues.[5] In contrast, synthesis of the four-membered heterocyclic amino acid, norproline (2-carboxyazetidine derivative) is underdeveloped,[6] which may be attributed to the lack of efficient synthetic methodologies for the preparation of functionalized azetidines, especially in enantiomerically pure forms.[7] In this paper, we report a new synthetic route towards azetidinic amino acids of general structure depicted in Fig. 1, in which the nature of the R group located at C-4 comes from enantiomerically pure β-amino alcohols.[8] This synthesis relies on an unprecedented intramolecular Michael addition to form the four-membered ring through carbon–carbon bond formation. The azetidinic amino acids, **35–44**, prepared *via* this novel methodology may be envisioned as C-4 alkyl substituted analogues of *trans*-2-carboxyazetidine-3-acetic acid (*t*-CAA)[9] and/or conformationally constrained analogues of (*R*)- or (*S*)-glutamic acid (Glu), the latter being the major excitatory neurotransmitter in the central nervous system (CNS).[10] Activation of the glutaminergic neurotransmitter system in the CNS is involved in important neurophysiological processes such as memory and learning, motor functions, and neural plasticity and development. However in the diseased brain, it is believed that neurodegenerative diseases such as Alzheimer's disease, dementia, Huntington's disease, amyotrophic lateral sclerosis (ALS), epilepsy and cerebral stroke may be directly related to disordered glutamatergic neurotransmission originating from dysfunction of either the Glu receptors (iGluR or mGluR) and/or the Glu re-uptake system (EAATs).[10,11] In the ongoing process to characterize and understand the function of the glutaminergic neurotransmitter system in the CNS, we have characterized the synthesized azetidinic amino acids, **35–44** as ligands at the ionotropic Glu receptors (iGluR), the metabotropic Glu receptors (mGluR) and the Glu transporters (EAAT1–3),[12,13] and present results, including a structure–activity study (SAR) in the second part of this paper.

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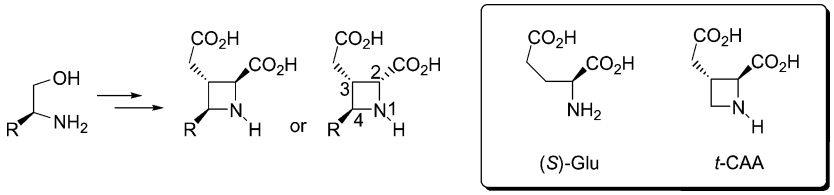

Fig. 1

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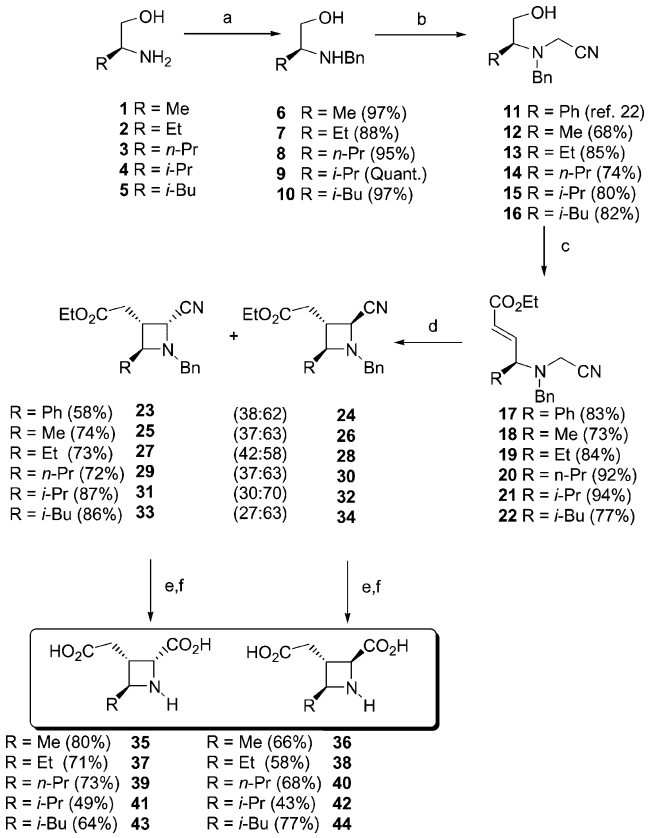
Results and discussion

Chemistry

Azetidinic amino acids **35–44** were prepared through a straightforward five step sequence starting from commercially available (*S*)-β-amino alcohols **1–5** possessing an alkyl substituent of various size. The first three steps include: (i) a *N*-benzylation through reductive amination of benzaldehyde to give **6–10**, (ii) an alkylation with bromoacetonitrile yielding **12–16** (compound **11** was previously reported),[14] (iii) a one-pot two-step Swern oxidation and Wittig olefination giving the unsaturated amino esters **17–22**.[15] The key step of this synthetic sequence is an original anionic 4-*exo*-tet ring closure that forms the azetidine ring upon treatment of these compounds with LiHMDS.[16] This reaction affords a mixture of 2,3-*cis* and 2,3-*trans* isomers in a 3 : 7 to 4 : 6 ratio. These diastereoisomers were easily separated by chromatography and were finally submitted to acidic hydrolysis followed by hydrogenolysis of the *N*-benzyl protecting group. Purification through ion-exchange chromatography yielded the desired amino acids **35–44** as white solids.

The relative configuration of the substituents in azetidines **25** and **26** was determined on the basis of NOE experiments. In fact, the examination of the values of ^3J coupling constants in these heterocycles does not give clear cut information about the relative stereochemistries. In the case of azetidines **23** and **24**, the relative configurations were determined by X-ray crystallography performed on **23**.[17] The relative configurations in the other compounds were deduced from (i) the chemical shift of the proton located at C-2, that constantly appears more deshielded of nearly 1 ppm in the *cis*-isomers than in the *trans*-isomers, (ii) the diastereomeric distribution of the products since the *cis*-isomer is always the minor one, and, (iii) the biological activity of the amino acid, the *cis*-isomer, presenting the unnatural (*R*) absolute configuration for the amino acid moiety being not active (*vide supra*) (Scheme 1).

The diastereoselectivity of this intramolecular Michael addition deserves comment. First of all, we were able to get some experimental evidence for thermodynamic control operating in the cyclization step. Indeed, when diastereoisomerically pure compounds **25** or **26** were treated under the same conditions used for ring closure (LiHMDS, −78 to 0 °C), an equilibration occurred that gave in both cases a mixture of **25** and **26** in a ratio almost identical to the one observed in the initial ring closure. Furthermore, lithiation of **25** at −78 °C immediately followed by deuteration with CD_3OD gave α-deuterated ester at the methylene position exclusively. These observations suggest the occurrence of a retro-Michael process that induces a base-catalyzed equilibration between **25** and **26**. Secondly, the exclusive formation of 3,4-*trans* isomers in these ring closures can be explained by considering the different transition states of this reaction. All of them involving substrate **18** (R = Me) are depicted in Fig. 2. In the course of the cyclization following



Scheme 1 *Reagents and conditions*: (a) PhCHO, 4 Å MS, CH_2Cl_2 then $NaBH_4$, EtOH (b) bromoacetonitrile, K_2CO_3, CH_3CN (c) DMSO, $(COCl)_2$, Et_3N, CH_2Cl_2, −78 °C, then $(C_6H_5)_3P{=}CHCO_2Et$ (d) LiHMDS, THF, −78 °C to 0 °C (e) 6N HCl–CCl_4 (f) H_2, Pd/C cat., EtOH.

transition state **A**, a severe $A^{1,3}$ strain is developed between the methyl substituent of the azetidine ring and the olefinic proton of the unsaturated ester, forbidding this pathway. This is not the case in transition state **B**, leading to **26**. A similar $A^{1,3}$ strain occurs in **C** but not in **D**, leading to **25**, the second isomer being formed in the reaction. To summarize, cyclization would proceed through transition states **B** and **D**, leading to **25** and **26**, which are in equilibrium.

Attempts to improve the diastereoselectivity of this cyclization in favor of the 2,3-*trans* isomer, presenting the (*S*)-configuration at C-2 were also done. The stereochemistry of the alkene Michael acceptor in **18** was first switched to (*Z*) using Still–Gennari methodology,[18] but the cyclization step occurred for this substrate with similar yields and diastereoselectivity, which is in accordance with the reversibility of the mechanism. A more encouraging result was obtained by replacing the nitrile by a *tert*-butyl ester. Thus, compound **46**, prepared in a similar way

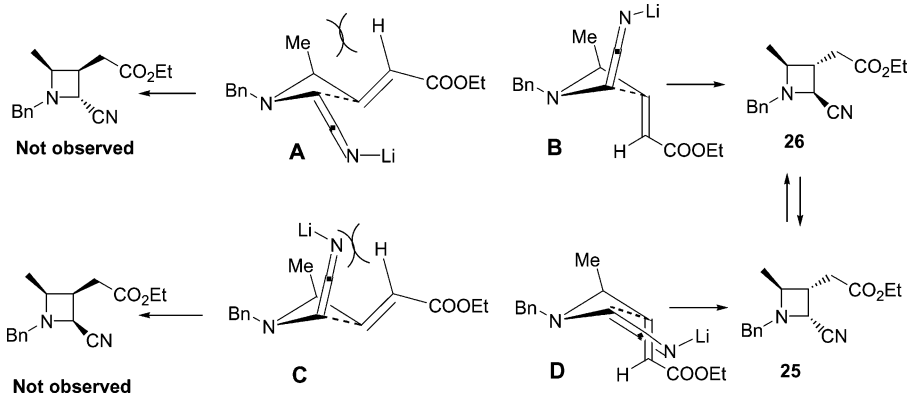
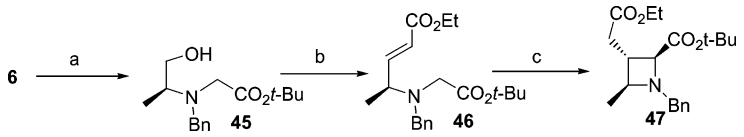


Fig. 2 Possible transition states for the anionic ring-closure.



Scheme 2 *Reagents and conditions*: (a) NaI, *tert*-butylbromoacetate, DMF, rt, 76% (b) DMSO, $(COCl)_2$, Et_3N, CH_2Cl_2, −78 °C, then $(C_6H_5)_3P{=}CHCO_2Et$, 77% (c) *t*-BuOK, THF, −50 °C, 50% (de >95%).

as described for the amino nitrile cyclized upon treatment with *t*-BuOK at −50 °C in THF to give a unique 2,3-*trans* isomer **47**, albeit with a modest yield of 50% (Scheme 2).

Pharmacology and SAR at iGlu and mGlu receptors

The pharmacological properties of the C-4 substituted azetidine analogues **35–44** were characterized in binding studies on native ionotropic Glu receptors and in functional assays at cloned metabotropic receptors mGluR1, 2 and 4, representing group I–III mGlu receptors, respectively (Table 1). The (2*S*)-azetidine analogues **36**, **38**, **40**, **42** and **44** exhibited low affinity for α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) preferring iGlu receptors (IC_{50} >100 μM). Affinities for the kainite (KAIN) preferring iGlu receptors were in the mid-to-low micro molar range, decreasing as the C-4 substituent was extended from a Me group, compound **36**, (IC_{50} = 5 μM) to an *n*-Pr group, compound **40**, (IC_{50} = 25 μM). Furthermore, the short but more bulky substituent *i*-Pr, compound **42**, showed mid-range affinity (IC_{50} = 6.8 μM) equivalent to compound **36** (R = Me), while the longer and more bulky substituent *i*-Bu, compound **44**, resulted in loss of binding affinity (IC_{50} >100 μM). At the *N*-methyl-D-aspartate (NMDA) preferring group of iGlu receptors, compounds **36**, **38** and **40** showed decreasing affinity in the μM mid-range, while the two compounds with bulky substituents, **42** and **44**, both exhibited low affinity. In the functional mGlu assays, the (2*S*)-4-Me-azetidine analogue **36** was characterized as an agonist, with weak potency (EC_{50} = 220 μM) at mGluR2 (group II) and neglectable potency (EC_{50} >1000 μM) at mGluR1 and 4.

We then conducted a SAR study to explain the observed pharmacological profile of the azetidine analogues at the iGlu and mGlu receptors. It is well-accepted that Glu enters a *folded* conformation when agonizing the AMPA and KAIN preferring iGlu receptors,[19] while an *extended* conformation of Glu is the agonist conformation at the mGlu receptors.[20] First, we submitted the compounds *t*-CAA, **36**, **38**, **40**, **42** and **44** to a stochastic conformational search in the modelling software program MOE (Table 2). An *extended* Glu conformation was identified as the low-energy conformation for the parental azetidinic system *t*-CAA and its C-4 analogues **36**, **38**, **40** and **44**, whereas the C-4 azetidine analogue **42** was forced into a *folded* Glu conformation, due to its small and bulky C-4 substituent (*i*-Pr). To fit the low-energy *extended* conformation of *t*-CAA, **36**, **38**, **40** and **44** to the respective *folded* Glu conformation, a systematic rotation of the 3-carboxymethyl group was carried out. This suggested a very small energy difference (average +0.49 kcal mol^{-1}) between the low-energy *extended* conformation and the respective *folded* conformations. Secondly, we wanted to investigate the flexibility of the R group at C-4. This was done by a systematic rotation of the C–C bonds of this group, keeping the conformation of the ammonium and two carboxylate groups fixed in the *folded* conformation. The number of structures within +1 kcal mol^{-1} were counted and the results are summarized in Table 2. We conclude that the degree of flexibility increases with increasing length of the R group (Me to *n*-Pr), whereas the small and bulky substituent, *i*-Pr, is locked into one position in space and the *i*-Bu is predicted to be less flexible, as compared with the *n*-Pr group.

We next wanted to address the affinity differences observed for compounds *t*-CAA and its 4-Me analogue **36** at the AMPA preferring receptors. The low-energy *folded* conformations of the two molecules were superimposed on KAIN in the iGluR2–S1S2 domain construct (PDB file name: 1FTK, crystallized with KAIN) (Fig. 3), by fitting the ammonium group and the two carboxylate groups. It can be seen that the ammonium groups

Table 1 Pharmacological evaluation of (2*S*)-azetidine analogues **36**, **38**, **40**, **42** and **44** at iGlu receptors. Data for (2*R*)-azetidine analogues **35**, **37**, **39**, **41** and **43** (IC_{50} >100 μM in AMPA, KAIN and NMDA assays, and **35** only: EC_{50} >1000 μM in mGluR1, 2 and 4 assays) omitted for clarity

	R	[^{3}H]AMPA IC_{50}/μM	[^{3}H]KAIN IC_{50}/μM	[^{3}H]CGP39653 K_i/μM	mGluR1 EC_{50}/μM	mGluR2 EC_{50}/μM	mGluR4 EC_{50}/μM
t-CAA	H	13	0.7	49[a]	210	>600	>300
36	Me	>100	5	2	>1000	220	>1000
38	Et	>100	7.5	8.7	n.t.	n.t.	n.t.
40	*n*-Pr	>100	25	22	n.t.	n.t.	n.t.
42	*i*-Pr	>100	6.8	>100	n.t.	n.t.	n.t.
44	*i*-Bu	>100	>100	>100	n.t.	n.t.	n.t.

[a] The ligand [^{3}H]CCP was used and IC_{50} value reported. n.t. = not tested.

Table 2 Modelling study of (2*S*)-azetidine analogues **36**, **38**, **40**, **42** and **44**. Identification of biologically relevant low-energy conformations

	R	Low-energy conformation ΔG/kcal mol^{-1}[a]	Folded conformation $\Delta\Delta G$/kcal mol^{-1}[b]	No. of R group conformations at $\Delta\Delta G$ + 1/kcal mol^{-1}[c]
t-CAA	H	−121.45	+1.02	0
36	Me	−117.97	+0.36	1
38	Et	−118.93	+0.40	2
40	*n*-Pr	−118.71	+0.34	4
42	*i*-Pr	−113.09	−1.78	1
44	*i*-Bu	−113.00	+0.34	2

[a] Located *via* a stochastic conformational search. [b] Systematic rotation of the distal carboxymethyl group starting with the molecule in its low-energy conformation. [c] Systematic rotation of the R group, starting with the molecule in its folded conformation. See experimental section for details.

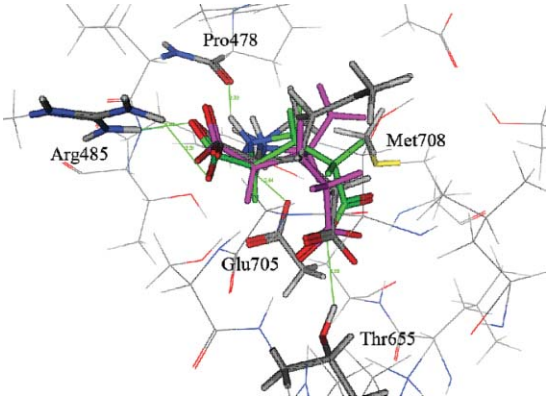


Fig. 3 Superimposition of *t*-CAA (green) and **36** (purple) with KAIN (type code) crystallized in iGluR2 (Brookhaven PDB-file name 1FTK), by fitting the ammonium group and the two carboxylate groups.

and the carboxylate groups of *t*-CAA and **36** are in favourable positions to interact with the amino acid residues, essential for agonist activity (Arg485, Pro478, Thr655 and Glu705). However for compound **36**, an unfavourable steric interaction can also be observed between the 4-Me group and the amino acid Met708, which we believe is the main reason for the observed low-affinities of **36** (and thus **38**, **40**, **42** and **44**) at the AMPA preferring Glu receptors.

Even though the azetidinic amino acids *t*-CAA, **36**, **38**, **40**, **42** and **44** readily adopt the *extended* conformation, required for agonist activity at the mGlu receptors, no potency was observed (Table 1). To address this finding, we did a superimposition study of *t*-CAA and **36** with Glu in the mGluR1 crystal structure (PDB file name: 1EWK, crystallized with Glu), by fitting the ammonium group and the two carboxylate groups (Fig. 4). The compounds overlaid well with Glu in the crystal structure, however with two critical steric conflicts between the azetidic C-4 and amino acid residue Asp318, and the C-4 substituent and the amino acid residue Lys409. As these two amino acids residues are conserved throughout the mGlu receptor subtypes, we believe that the before mentioned disfavoured van der Waals interactions are the major reasons for the observed lack of activity at the mGlu receptors.

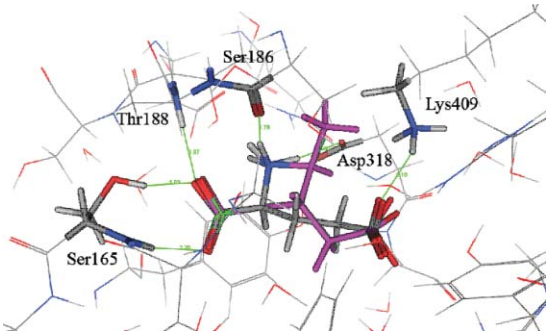


Fig. 4 Superimposition of *t*-CAA (green) and **36** (purple) with Glu (type code) crystallized in mGluR1 (Brookhaven PDB-file name 1EWK), by fitting the ammonium group and the two carboxylate groups.

Pharmacology and SAR at Glu transporters

The azetidinic amino acid analogues **35**, **36** and **40** were evaluated as ligands for the glutamate transporters EAAT1–3. While the (2*R*)-azetidine analogue, compound **35**, was inactive, (2*S*)-azetidines **36** and **40** were found to be inhibitors at EAAT1–3, with neglectable potency at subtype EAAT1, medium potency at subtype EAAT2, equivalent to dihydrokainic acid (DHK), and low potency at subtype EAAT3, decreasing as the C-4 substituent is extended from a Me group to an *n*-Pr group (Table 3). In contrast to the non-selective ligand *t*-CAA, which holds the azetidinic parental system, we observe selectivity for the EAAT2 subtype, upon stereospecific introduction of a (4*S*)-4-alkyl substituent. In fact we see increasing selectivity for subtype EAAT2 over subtype EAAT3 (ratio 4 *vs.* 8), as the alkyl substituent is extended to an *n*-Pr group.

It has been suggested by us[21] and others[22–24] that the inhibitory binding conformation at EAAT1–3 is similar to the *folded* Glu conformation also observed for iGluR agonist activity, while substrates bind in an *extended* Glu conformation, however different from the conformation observed for mGluR agonist activation. Selective inhibition of the EAAT2 subtype has previously been observed for the natural product KAIN as well as its close structural analogue DHK. We therefore performed a superimposition study of the four low-energy *folded* conformations found for azetidine analogue **40** with the low-energy conformation of KAIN, to investigate the degree of overlap of the two substituents (Fig. 5). However, we see that the *n*-Pr group at the best is at the brink of the isopropenyl group in KAIN, allowing us to suggest that the EAAT2 transporter protein may be able to accommodate larger substituents than an isopropenyl group. We believe that the synthesis of other azetidinic amino acids with longer and more bulky alkyl substituents in the 4 position would be beneficial in the search for potent selective EAAT2 subtype inhibitors.

Table 3 Pharmacological evaluation of azetidine analogues **35**, **36** and **40** at Glu transporters. Data for KAIN and DHK taken from ref. 31; data for *t*-CAA taken from ref. 9

	R	EAAT1 K_i/μM	EAAT2 K_i/μM	EAAT3 K_i/μM
KAIN	—	>3000	60	>3000
DHK	—	>3000	31	>3000
t-CAA	H	5	5	5
(2*R*)-**35**	Me	>1000	>1000	>1000
(2*S*)-**36**	Me	>1000	32	126
(2*S*)-**40**	*n*-Pr	>1000	44	375

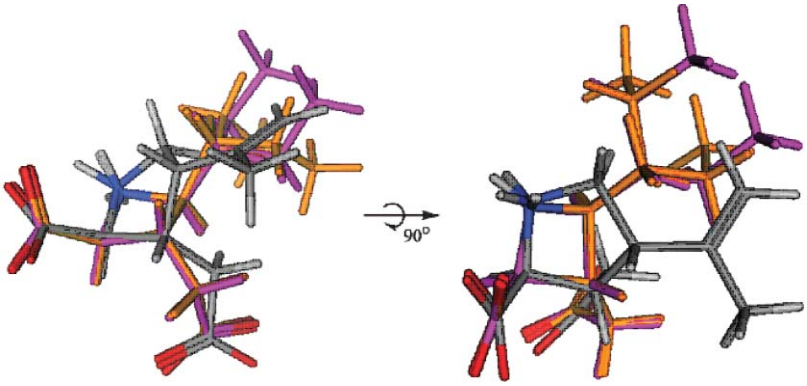


Fig. 5 Superimposition of the low-energy conformation of KAIN (type code) and four low-energy conformations of **40** (purple and orange, for clarity), by fitting the ammonium group and the two carboxylate groups.

Introduction of a methyl substituent in the amino acid's α′ position has previously been done, maintaining potency at the EAATs.[25] Thus, in rat forebrain synaptosomes, an EAAT subtype non-specific assay, the amino acids L-*trans*-2,3-PDC and its *cis*-5-methyl derivative (Fig. 7) were shown to inhibit D-[^{3}H]aspartate uptake by 70%. On the other hand, the C-5 diastereomer *trans*-5-methyl-L-*trans*-2,3-PDC (Fig. 7) did not inhibit D-[^{3}H]aspartate uptake. We decided to overlay low-energy conformations of *cis*-5-methyl-L-*trans*-2,3-PDC with our azetidine analogue **36** to investigate the orientation of the two methyl groups in space (Fig. 6). It can be seen that the two methyl groups are positioned very closely in space pointing in the same direction. This superimposition study also provides the information that the C-4 diastereomers of our azetidine analogues are likely not ligands at the EAATs.

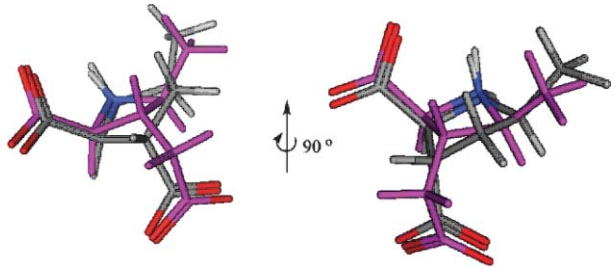


Fig. 6 Superimposition of low-energy conformations of *cis*-5-Me-L-*trans*-2,3-PDC (type code) and **36** (purple) by fitting the ammonium group and the two carboxylate groups.

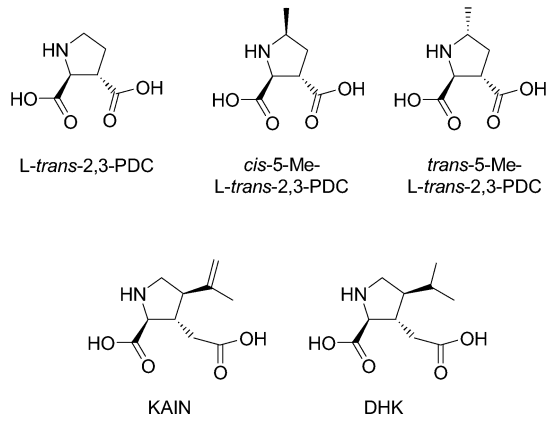


Fig. 7 Known EAAT inhibitors.

In conclusion, for the synthetic part, we have discovered a very efficient synthetic methodology for the construction of a functionalized azetidine ring that allows for a straightforward preparation of an understudied class of cyclic amino acids. The nature of the thermodynamic driving force overriding the developing torsional strain in the four-membered ring produced during the key anionic ring closure remains to be fully understood but the efficiency of this challenging reaction contributes to putting into perspective the preconceived strain in azetidinic heterocycles.

The ten synthesized novel conformationally restricted Glu analogues **35–44** were characterized in binding studies on native ionotropic Glu receptors and in functional assays at cloned metabotropic receptors mGluR1, 2 and 4, representing group I–III mGlu receptors, respectively. The (2*R*)-azetidines **35**, **37**, **39**, **41** and **43** were all inactive, whereas a marked change in the pharmacological profile at the iGlu receptors was observed when a methyl group was introduced in the C-4 position, compound **36** *versus t*-CAA. However, within the series of azetidine analogues presented here, no receptor-class nor subgroup selectivity could be demonstrated. Azetidine analogues **35**, **36** and **40** were also characterized as potential ligands at the glutamate transporter subtypes EAAT1–3 in the FLIPR® Membrane Potential (FMP) assay. While **35** was inactive, azetidines **36** and **40** were both identified as inhibitors and showed selectivity for the EAAT2 subtype. On the basis of the new pharmacological data obtained, the modelling study provided new insight into the SAR of the glutamate receptors and transporters.

Experimental

Chemistry

General comments. ^{1}H and ^{13}C spectra ($CDCl_3$ solution unless otherwise stated) were recorded on a Bruker AC 200 or 300 spectrometer at 200, 300 (^{1}H), 50.3 and 75.5 (^{13}C) MHz; chemical shifts are reported in ppm from TMS. Optical rotations were determined with a Perkin Elmer 241 instrument. All reactions were carried out under argon. Column chromatography was performed on silica gel 230–400 mesh by using various mixtures of diethyl ether (E), ethyl acetate (AcOEt), cyclohexane (CyH) and petroleum ether (PE). TLC was run on Merck Kieselgel $60F_{254}$ plates. Melting points are uncorrected. THF was distilled from sodium–benzophenone ketyl. Dichloromethane and triethylamine were distilled from calcium hydride. Benzaldehyde was distilled before use. Other reagents were used as purchased. Mention of "usual workup" means: (i) decantation of the organic layer, (ii) extraction of the aqueous layer with ether, (iii) washing the combined organic layers with brine and drying of the combined organic phases over $MgSO_4$, (iv) solvent evaporation under reduced pressure. Compositions of stereoisomeric mixtures were determined by NMR analysis on crude products before any purification. Elementary analyses were realized by "Le service régional de microanalyse de l'Université de pharmacie de Châtenay-Malabry". HRMS was performed on a Finnagan MAT-90 spectrometer at Boston University and in the laboratory of "Ecole Nationale Supérieure de Paris" (compounds **35** and **36**). Mass spectra were recorded on a GC-MS HP MS 5989B spectrometer at the University of Versailles.

General procedure for the *N*-benzylation of (*S*)-amino alcohols

To a solution of the β-amino alcohol (26 mmol) in dichloromethane (50 mL) was added 4 Å molecular sieves (5 g) and benzaldehyde (2.60 mL, 26 mmol). After 3 h at rt without stirring, the suspension was filtered and concentrated under reduced pressure. The residue was dissolved in EtOH (50 mL) and sodium borohydride was added portionwise (1.16 g, 31 mmol). After 2 h at rt, the reaction was hydrolyzed by addition of a saturated aqueous solution of NH_4Cl and concentrated under reduced pressure. Basification of the aqueous residue with 1N NaOH was followed by the usual workup and gave crude *N*-benzyl amino alcohols that were used without further purification for the next step.

(*S*)-*N*-Benzyl-2-aminopropan-1-ol 6. Yield: 63%; R_f: 0.12 (E–30% aqueous ammonia, 98 : 2); mp 38–42 °C; $[\alpha]_D^{20}$ +58.9 (*c* 1.0, $CHCl_3$); δ_H(250 MHz, $CDCl_3$, Me_4Si): 1.34 (d, 3H, J = 6.5, Me), 2.81 (s, 2H, NH and OH), 3.09 (quintd, 1H, J = 4.0, 6.5, C*H*CH$_3$), 3.56 (dd, 1H, J = 7.1, 10.8, C*H*HOH), 3.84 (dd, 1H, J = 4.0, 10.8, CH*H*OH), 3.98 (d, 1H, J = 12.9, C*H*HPh), 4.13 (d, 1H, J = 12.9, CH*H*Ph), 7.51–7.61 (m, 5H, Ph); δ_C(62.9 MHz, $CDCl_3$, Me_4Si): 17.0 (CH_3), 51.1 (CH_2), 53.8 (CH), 65.8 (CH_2), 127.2, 128.2, 128.5 (CHAr), 140.1 (CqAr).

(*S*)-*N*-Benzyl-2-aminobutan-1-ol 7. Yield: 88%; R_f: 0.4 (AcOEt); mp 72 °C; $[\alpha]_D^{20}$ +28.0 (*c* 1.2, $CHCl_3$); δ_H(300 MHz, $CDCl_3$, Me_4Si): 0.85 (t, 3H, J = 7.3, Me), 1.42–1.50 (m, 2H, C*H*$_2$CH$_3$), 2.18 (bs, 2H, NH and OH), 2.50–2.58 (m, 1H, C*H*CH$_2$), 3.26 (d, 1H, J = 6.3, C*H*HOH), 3.57 (d, 1H, J = 6.3, CH*H*OH), 3.67 (d, 1H, J = 12.9, C*H*HPh), 3.75 (d, 1H, J = 12.9, CH*H*Ph), 7.16–7.28 (m, 5H, Ph); δ_C(75 MHz, $CDCl_3$, Me_4Si): 10.4 (CH_3), 24.2, 51.0 (CH_2), 59.8 (CH), 62.6 (CH_2), 127.02, 128.1, 128.5 (CHAr), 140.3 (CqAr); MS (CI, CH_4): *m*/*z* 180 (MH^+), 90.

(*S*)-*N*-Benzyl-2-aminopentan-1-ol 8. Yield: 95%; R_f: 0.21 (Et_2O); $[\alpha]_D^{20}$ +20 (*c* 1.1, $CHCl_3$); δ_H(300 MHz, $CDCl_3$, Me_4Si): 0.93 (t, 3H, *J* = 7.3, Me), 1.25–1.52 (m, 4H, $(CH_2)_2CH_3$), 2.61–2.69 (bm, 3H, NH, OH, $CHCH_2$), 3.33 (dd, 1H, *J* = 6.6 and 11, $CHHOH$), 3.63 (d, 1H, *J* = 3.9 and 11, $CHHOH$), 3.75 (A part of an AB syst., 1H, *J* = 12.7, $CHHPh$), 3.82 (B part of a AB syst., 1H, *J* = 12.9, $CHHPh$), 7.11–7.23 (m, 5H, Ph); δ_C(75 MHz, $CDCl_3$, Me_4Si): 14.3 (CH_3), 19.3, 33.7, 51.4 (CH_2), 58.1 (CH), 63.0 (CH_2), 127.2, 128.1, 128.6 (CHAr), 140.7 (CqAr); MS (CI, CH_4): *m*/*z* 194 (MH^+).

(*S*)-*N*-Benzyl valinol 9. Yield: quant.; R_f: 0.4 (AcOEt); $[\alpha]_D^{20}$ +16.0 (*c* 1.4, $CHCl_3$); δ_H(300 MHz, $CDCl_3$, Me_4Si): 0.93 and 0.98 (two d, 6H, *J* = 6.8, Me), 1.84–1.94 (m, 1H, $CH(CH_3)_2$), 2.47–2.53 (bm, 3H, NH, OH, $CHCHMe_2$), 3.42 (dd, 1H, *J* = 3.5 and 11, $CHHOH$), 3.67 (dd, 1H, *J* = 6.3 and 11, $CHHOH$), 3.80 (s, 2H, CH_2Ph), 7.18–7.30 (m, 5H, Ph); δ_C(75 MHz, $CDCl_3$, Me_4Si): 18.6, 19.5 (CH_3), 28.9 (CH), 51.5, 60.5 (CH_2), 77.2 (CH), 127.0, 128.5, 128.7 (CHAr), 140.4 (CqAr); MS (CI, CH_4): *m*/*z* 194 (MH^+, 18), 193 (100), 162, 91.

(*S*)-*N*-Benzyl leucinol 10. Yield: 97%; R_f: 0.45 (AcOEt); mp 70 °C; $[\alpha]_D^{20}$ +31.0 (*c* 1.0, $CHCl_3$); δ_H(200 MHz, $CDCl_3$, Me_4Si): 0.89 and 0.92 (two d, 6H, *J* = 6.8, Me), 1.19–1.50 (m, 1H, $CH(CH_3)_2$), 1.58–1.68 (m, 2H, $CH_2CH(Me)_2$), 2.13 (bs, 2H, NH and OH), 2.73–2.80 (m, 1H, CHN), 3.29 (d, 1H, *J* = 11, $CHHOH$), 3.42 (d, 1H, *J* = 11, $CHHOH$), 3.81 (d, *J* = 2.4, 2H, CH_2Ph), 7.27–7.36 (m, 5H, Ph); δ_C(75 MHz, $CDCl_3$, Me_4Si): 22.7, 23.1 (CH_3), 25.0 (CH), 40.9, 50.8, 56.1 (CH_2), 63.0 (CH), 127.0, 128.0, 128.3 (CHAr), 139.5 (CqAr); MS (CI, NH_3): *m*/*z* 221.2, 220.3, 206.4, 162.7, 91.9; Anal. calcd. for $C_{13}H_{21}NO$: C, 75.32; H, 10.21; N, 6.76. Found: C, 75.28; H, 10.32; N, 6.65%.

General procedure for alkylation with bromoacetonitrile

To a solution of *N*-benzyl aminoalcohol (9.66 mmol) in acetonitrile (80 mL) was added potassium carbonate (2.06 g, 14.9 mmol) and bromoacetonitrile (1.90 mL, 27 mmol). The resulting mixture was refluxed for 2.5 h and, after cooling to rt, concentrated to dryness under reduced pressure. After addition of water and ether, the usual workup was followed by flash chromatography (AcOEt–CyH, 2 : 8) of the crude residue and gave the following compounds:

(*S*)-*N*-Benzyl-*N*-cyanomethyl-2-aminopropan-1-ol 12. Yield: 68%; R_f: 0.34 (E–CyH, 8 : 2); $[\alpha]_D^{25}$ +75.2 (*c* 1.5, $CHCl_3$); IR (film): 3441, 2965, 2930, 2879, 2228, 1496, 1445 cm^{-1}; δ_H(250 MHz, $CDCl_3$, Me_4Si): 0.98 (d, 3H, *J* = 6.8, Me), 2.39 (bs, 1H, OH), 2.81–2.96 (m, 1H, CHMe), 3.22 (d, 2H, *J* = 5.9, CH_2CN), 3.31 (d, 2H, *J* = 6.2, CH_2OH), 3.48 (d, 1H, *J* = 13.3, $CHHPh$), 3.66 (d, 1H, *J* = 13.3, $CHHPh$), 7.05–7.15 (m, 5H, Ph); δ_C(62.9 MHz, $CDCl_3$, Me_4Si): 11.8 (CH_3), 38.5, 53.1 (CH_2), 59.6 (CH), 63.5 (CH_2), 116.8 (CN), 128.0, 128.9, 129.0 (CHAr), 136.9 (CqAr); Anal. Calcd. for $C_{12}H_{16}N_2O$: C, 70.56; H, 7.89; N, 13.71. Found: C, 69.78; H, 7.90; N, 13.32%.

(*S*)-*N*-Benzyl-*N*-cyanomethyl-2-aminobutan-1-ol 13. Yield: 85%; R_f: 0.85 (AcOEt–PE, 1 : 1); $[\alpha]_D^{20}$ +35.0 (*c* 1.1, $CHCl_3$); δ_H(300 MHz, $CDCl_3$, Me_4Si): 0.99 (t, 3H, *J* = 7.2, Me), 1.36–1.92 (m, 2H, CH_2CH_3), 2.70 (bs, 1H, OH), 2.76–2.87 (m, 1H, $CHCH_2$), 3.46–3.70 (m, 4H, CH_2CN and CH_2OH), 3.78 (d, 1H, *J* = 13.4, $CHHPh$), 3.91 (d, 1H, *J* = 13.3, $CHHPh$), 7.15–7.45 (m, 5H, Ph); δ_C(62.9 MHz, $CDCl_3$, Me_4Si): 11.5 (CH_3), 20.2, 38.2, 53.9 (CH_2), 61.2 (CH), 65.9 (CH_2), 117.1 (CN), 127.9, 128.5, 128.9 (CHAr), 137.2 (CqAr); MS (CI, NH_3): *m*/*z* 192.6, 162.8, 92.5; Anal. Calcd. for $C_{13}H_{18}N_2O$: C, 71.53; H, 8.31; N, 12.83. Found: C, 71.60; H, 8.39; N, 12.72%.

(*S*)-*N*-Benzyl-*N*-cyanomethyl-2-aminopentan-1-ol 14. Yield: 74%; R_f: 0.89 (AcOEt–PE, 1 : 1); $[\alpha]_D^{20}$ +28.0 (*c* 1.1, $CHCl_3$); δ_H(300 MHz, $CDCl_3$, Me_4Si): 0.96 (t, 3H, *J* = 7.2, Me), 1.27–1.41 (m, 4H, $(CH_2)_2CH_3$), 2.58 (bs, 1H, OH), 2.91–3.01 (m, 1H, CHN), 3.46 (s, 2H, CH_2CN), 3.59 (dd, 1H, *J* = 6.6 and 11, $CHHOH$), 3.69 (dd, 1H, *J* = 3.7 and 11, $CHHOH$), 3.76 (d, 1H, *J* = 13.4, $CHHPh$), 3.92 (d, 1H, *J* = 13.3, $CHHPh$), 7.30–7.34 (m, 5H, Ph); δ_C(62.9 MHz, $CDCl_3$, Me_4Si): 14.2 (CH_3), 20.1, 29.1, 38.2, 53.9, 61.6 (CH_2), 64.1 (CH), 117.0 (CN), 127.4, 127.9, 128.9 (CHAr), 137.2 (CqAr).

(*S*)-*N*-Benzyl-*N*-cyanomethyl-valinol 15. Yield: 80%; R_f: 0.77 (AcOEt); $[\alpha]_D^{20}$ −9 (*c* 1.6, $CHCl_3$); δ_H(300 MHz, $CDCl_3$, Me_4Si): 0.99 (t, 3H, *J* = 6.8, Me), 1.11 (t, 3H, *J* = 6.8, Me), 1.96–2.08 (m, 1H, $CH(CH_3)_2$), 2.35 (bs, 1H, OH), 2.53–2.60 (m, 1H, $CHCHMe_2$), 3.49 (d, 1H, *J* = 17.5, $CHHOH$), 3.54 (d, 1H, *J* = 17.5, $CHHOH$), 3.72–3.98 (m, 4H, CH_2Ph, CH_2CN), 7.28–7.39 (m, 5H, Ph); δ_C(62.9 MHz, $CDCl_3$, Me_4Si): 19.8, 21.5 (CH_3), 27.6 (CH), 38.4, 55.9, 59.8 (CH_2), 69.8 (CH), 117.4 (CN), 127.0, 128.5, 128.9 (CHAr), 137.8 (CqAr); MS (CI, NH_3): *m*/*z* 206.4, 205.3, 162.3, 90.9; Anal. Calcd. for $C_{14}H_{20}N_2O$: C, 72.38; H, 8.68; N, 12.06. Found: C, 72.12; H, 8.84; N, 11.95%.

(*S*)-*N*-Benzyl-*N*-cyanomethyl-leucinol 16. Yield: 82%; R_f: 0.33 (E–CyH, 1 : 1); $[\alpha]_D^{20}$ +51 (*c* 1.5, $CHCl_3$); δ_H(200 MHz, $CDCl_3$, Me_4Si): 0.97 (d, 6H, *J* = 6.1, 2Me), 1.21–1.34 (m, $CH(Me)_2$), 1.58–1.74 (m, 2H, $CHCH_2CH$), 2.52 (bs, 1H, OH), 2.99–3.09 (m, 1H, CHN), 3.47–3.71 (m, 4H, CH_2OH, CH_2CN), 3.78 (d, 1H, *J* = 13.6, $CHHPh$), 3.78 (d, 1H, *J* = 13.6, $CHHPh$), 7.20–7.40 (m, 5H, Ph); δ_C(62.9 MHz, $CDCl_3$, Me_4Si): 22.3, 23.3 (CH_3), 25.4 (CH), 35.8, 38.2, 53.9 (CH_2), 61.2 (CH), 62.4 (CH_2), 116.9 (CN), 127.9, 128.9, 129.0 (CHAr), 137.2 (CqAr); MS (IC, NH_3): *m*/*z* 220.3, 162.7, 91.9.

General procedure for the "one-pot" Swern reaction and Wittig olefination

To a solution of oxalyl chloride (0.16 mL, 1.8 mmol) in dichloromethane (10 mL) was added dropwise at −50 °C DMSO (0.15 mL, 1.7 mmol). After five minutes, the reaction mixture was cooled to −78 °C and the amino alcohol (1.2 mmol) in dichloromethane (5 mL) was added dropwise. After 30 min at −78 °C, triethylamine (0.63 mL, 3.7 mmol) was added dropwise, followed after 5 minutes by a solution of carboethoxymethylen-etriphenylphosphorane (0.8 g, 2.3 mmol) in dichloromethane (5 mL). The reaction mixture was then gradually allowed to reach room temperature and was hydrolysed by addition of water. Usual workup (dichloromethane) gave a residue that was purified by flash chromatography.

(2*E*,4*S*)-4-(Benzyl-cyanomethyl-amino)-4-phenyl-but-2-enoic acid ethyl ester 17. Yield: 83%; R_f: 0.72 (E–CyH, 6 : 4); $[\alpha]_D^{25}$ −40 (*c* 0.9, $CHCl_3$); δ_H(250 MHz, $CDCl_3$, Me_4Si): 1.19 (t, 3H, *J* = 7.3, CH_3), 3.27 (d, 1H, *J* = 17.5, $CHHCN$), 3.28 (d, 1H, *J* = 17.5, $CHHCN$), 3.60 (AB syst., 2H, *J* = 13.5, CH_2Ph), 4.09 (q, 2H, *J* = 7.3, OCH_2), 4.23 (d, 1H, *J* = 9.3, $NCHPh$), 6.11 (d, 1H, *J* = 15.5, $CHCOOEt$), 6.96 (dd, 1H, *J* = 9.8, 15.8, $CH{=}CHCO_2Et$), 7.18–7.41 (m, 10H, Ph); δ_C(62.9 MHz, $CDCl_3$, Me_4Si): 14.3 (CH_3), 39.2, 55.6, 60.8 (CH_2), 69.6 (CH), 114.1 (CN), 123.4 (CH), 128.0, 128.1, 128.8, 128.9, 128.9, 129.5 (CHAr), 136.9, 139.0 (Cq Ar), 147.2 (CH), 166.0 (CO); Anal. Calcd. for $C_{21}H_{22}N_2O_2$: C, 75.42; H, 6.63; N, 8.38. Found: C, 74.93; H, 6.73; N, 8.02%.

(2*E*,4*S*)-4-(Benzyl-cyanomethyl-amino)-pent-2-enoic acid ethyl ester 18. Yield: 73%; R_f: 0.53 (E–CyH, 1 : 1); $[\alpha]_D^{25}$ −37.9 (*c* 0.9, $CHCl_3$); δ_H(250 MHz, $CDCl_3$, Me_4Si): 1.04 (t, 3H, *J* = 7.3, CH_3), 1.08 (d, 3H, *J* = 6.8, CH_3), 3.15 (AB syst., 2H, *J* = 17.6, CH_2CN), 3.18–3.32 (m, 1H, $CHCH_3$), 3.48 (AB syst., 2H, *J* = 17.6, CH_2Ph), 3.95 (q, 2H, *J* = 7.1, CH_2CH_3), 5.78 (dd, 1H, *J* = 1.0, 15.8, $CHCOOEt$), 6.66 (dd, 1H, *J* = 7.4, 15.7, $CH{=}CHCO_2Et$), 7.00–7.09 (m, 5H, Ph); δ_C(62.9 MHz, $CDCl_3$, Me_4Si): 14.3, 17.5 (CH_3), 38.5, 55.2, 60.7 (CH_2), 115.7 (CN), 123.0 (CH), 127.9, 128.8, 128.9 (CHAr), 137.0 (Cq Ar), 149.3 (CH), 166.2 (CO); Anal. Calcd. for $C_{16}H_{20}N_2O_2$: C, 70.56; H, 7.40; N, 10.29. Found: C, 70.64; H, 7.55; N, 10.18%.

(2*E*,4*S*)-4-(Benzyl-cyanomethyl-amino)-hex-2-enoic acid ethyl ester 19. Yield: 84%; R_f: 0.66 (AcOEt–PE, 1 : 1); $[\alpha]_D^{25}$ −37 (*c* 1.1, $CHCl_3$); δ_H(300 MHz, $CDCl_3$, Me_4Si): 0.95 (t, 3H, $J = 7.3$, CH_3), 1.31 (t, 3H, $J = 7.1$, CH_3), 1.57–1.58 (m, 2H, CH_2CH_3), 3.21–3.29 (m, 1H, CHN), 3.37 (d, 1H, $J = 17.9$, CHHCN), 3.45 (d, 1H, $J = 17.9$, CHHCN), 3.68 (d, 1H, $J = 13.5$, CHHPh), 3.80 (d, 1H, $J = 13.5$, CHHPh), 4.22 (q, 2H, $J = 7.0$, CH_2O), 6.03 (d, 1H, $J = 15.7$, CHCOOEt), 6.90 (dd, 1H, $J = 8.6$, 15.7, CH=$CHCO_2Et$), 7.27–7.34 (m, 5H, Ph); δ_C(62.9 MHz, $CDCl_3$, Me_4Si): 10.1, 14.2 (CH_3), 24.6, 38.5, 54.9, 60.6 (CH_2), 64.6 (CH), 115.7 (CN), 124.5 (CH), 127.8, 128.7, 128.8, 129.1 (CHAr), 137.1 (Cq Ar), 146.3 (CH), 170.0 (CO); MS (IC, NH_3): *m*/*z*: 286.6, 256.9, 91.9; Anal. Calcd. for $C_{17}H_{22}N_2O_2$: C, 71.30; H, 7.74; N, 9.78. Found: C, 71.29; H, 7.82; N, 9.76%.

(2*E*,4*S*)-4-(Benzyl-cyanomethyl-amino)-hept-2-enoic acid ethyl ester 20. Yield: 92%; R_f: 0.7 (AcOEt–PE, 1 : 1); $[\alpha]_D^{25}$ −37 (*c* 1.1, $CHCl_3$); δ_H(300 MHz, $CDCl_3$, Me_4Si): 0.95 (t, 3H, $J = 7.1$, CH_3), 1.30 (t, 3H, $J = 7.1$, CH_3), 1.31–1.90 (m, 4H, $(CH_2)_2CH_3$), 3.32–3.38 (m, 1H, CHN), 3.43 (s, 2H, CH_2CN), 3.68 (d, 1H, $J = 13.5$, CHHPh), 3.84 (d, 1H, $J = 13.5$, CHHPh), 4.24 (q, 2H, $J = 7.0$, CH_2O), 6.03 (d, 1H, $J = 14.2$, CHCOOEt), 6.90 (dd, 1H, $J = 8.6$, 14.7, CH=$CHCO_2Et$), 7.25–7.35 (m, 5H, Ph); δ_C(62.9 MHz, $CDCl_3$, Me_4Si): 14.0, 14.2 (CH_3), 19.0, 33.8, 38.5, 54.9, 60.6 (CH_2), 63.0 (CH), 115.8 (CN), 124.3 (CH), 127.8, 128.7, 128.8 (CHAr), 137.1 (Cq Ar), 146.5 (CH), 165.9 (CO).

(2*E*,4*S*)-4-(Benzyl-cyanomethyl-amino)-5-methyl-hex-2-enoic acid ethyl ester 21. Yield: 94%; R_f: 0.53 (AcOEt–PE, 2 : 8); $[\alpha]_D^{25}$ −32 (*c* 3.3, $CHCl_3$); δ_H(300 MHz, $CDCl_3$, Me_4Si): 0.91 (d, 3H, $J = 6.8$, CH_3), 1.01 (d, 3H, $J = 6.8$, CH_3), 1.35 (t, 3H, $J = 7.1$, CH_3), 2.03–2.10 (m, 1H, CHMe$_2$), 3.48 (dd, 1H, $J =$ 7.1 and 9.8, CHN), 3.35 (d, 1H, $J = 17.6$, CHHCN), 3.43 (d, 1H, $J = 17.6$, CHHCN), 3.43 (d, 1H, $J = 13.5$, CHHPh), 3.43 (d, 1H, $J = 13.5$, CHHPh), 4.22 (q, 2H, $J = 7.1$, OCH_2CH_3), 6.00 (d, 1H, $J = 15.8$, CHCOOEt), 6.85 (dd, 1H, $J = 10$ and 15.7, CH=$CHCO_2Et$), 7.22–7.35 (m, 5H, Ph); δ_C(62.9 MHz, $CDCl_3$, Me_4Si): 14.2, 17.8, 20.1 (CH_3), 28.7 (CH), 38.6, 54.9, 60.4 (CH_2), 69.2 (CH), 115.6 (CN), 125.9 (CH), 127.8, 128.7, 130.2 (CHAr), 137.1 (Cq Ar), 144.2 (CH), 165.5 (CO); MS (CI, NH_3): *m*/*z*: 301.2, 300.3, 257.3, 108.0, 90.9; Anal. Calcd. for $C_{18}H_{24}N_2O_2$: C, 71.97; H, 8.05; N, 9.33. Found: C, 71.98; H, 7.98; N, 9.27%.

(2*E*,4*S*)-4-(Benzyl-cyanomethyl-amino)-6-methyl-hept-2-enoic acid ethyl ester 22. Yield: 77%; R_f: 0.43 (E–CyH, 85 : 15); $[\alpha]_D^{25}$ −16 (*c* 1.1, $CHCl_3$); δ_H(300 MHz, $CDCl_3$, Me_4Si): 0.83 (d, 6H, $J = 6.8$, $2CH_3$), 1.31 (t, 3H, $J = 7.2$, CH_3), 1.40–2.69 (m, 3H, CH_2CHMe_2), 3.35–3.48 (m, 3H, CHN, CH_2CN), 3.66 (d, 1H, $J = 13.4$, CHHPh), 3.85 (d, 1H, $J = 13.4$, CHHPh), 4.23 (q, 2H, $J = 7.1$, OCH_2CH_3), 6.02 (d, 1H, $J = 16$, CHCOOEt), 6.89 (dd, 1H, $J = 8$ and 16, CH=$CHCO_2Et$), 7.21–7.35 (m, 5H, Ph); δ_C(62.9 MHz, $CDCl_3$, Me_4Si): 14.2, 22.1, 23.1 (CH_3), 24.6 (CH), 38.3, 40.5, 54.7, 60.6 (CH_2), 61.2 (CH), 116.6 (CN), 124.3 (CH), 127.8, 128.7, 130.2 (CHAr), 137.1 (Cq Ar), 146.2 (CH), 165.5 (CO); MS (CI, NH_3): *m*/*z*: 315.2, 314.3, 256.8, 92.4; Anal. Calcd. for $C_{19}H_{26}N_2O_2$: C, 72.58; H, 8.33; N, 8.91. Found: C, 72.69; H, 8.48; N, 8.80%.

General procedure for azetidine formation

To a solution of amino ester (1.19 mmol) in THF (5 mL) was added, at −78 °C, a solution of LiHMDS (1 M solution in THF, 1.42 mL, 1.42 mmol). The mixture was gradually warmed to −10 °C over a period of 2 h and the progress of the reaction was monitored by TLC. After completion, hydrolysis by a saturated aqueous solution of ammonium chloride was followed by the usual workup (AcOEt). Flash chromatography of the residue on silica gel (eluant: AcOEt–PE, 5 : 95 then 1 : 9) gave 2,3-*cis*-isomers, followed by 2,3-*trans*-isomers.

(3*R*,4*S*,5*R*)-(1-Benzyl-2-cyano-4-phenyl-azetidin-3-yl)-acetic acid ethyl ester 23. *R* = *Ph*: overall yield: 58%. *Cis–trans* ratio, 38 : 62.

Yield: 22%; R_f: 0.72 (E–CyH, 60 : 40); $[\alpha]_D^{25}$ +91.6 (*c* 0.4, $CHCl_3$); δ_H(250 MHz, $CDCl_3$, Me_4Si): 1.08 (t, 3H, $J = 7.5$, CH_3), 2.61 (dd, 1H, $J = 4.6$, 12.9, CHHCO_2Et), 2.73–2.98 (m, 2H, CHHCO_2Et and $CHCH_2$), 3.70 (AB syst., 2H, $J = 13.3$, CH_2Ph), 3.97 (brquad, 3H, $J = 7.1$, OCH_2 and CHPh), 4.34 (d, 1H, $J = 7.1$, CHCN), 7.18–7.38 (m, 10H, Ar); δ_C(62.9 MHz, $CDCl_3$, Me_4Si): 14.2 (CH_3), 34.2 (CH_2), 40.6, 55.3 (CH), 56.0, 61.0 (CH_2), 72.7 (CH), 115.7 (CN), 127.1, 127.7, 128.6, 128.8, 129.2 (CHAr), 136.5, 139.5 (CqAr), 170.7 (CO); Anal. Calcd. for $C_{21}H_{22}N_2O_2$: C, 75.42; H, 6.63; N, 8.38. Found: C, 74.93; H, 6.73; N, 8.02%.

(3*S*,4*R*,5*R*)-(1-Benzyl-2-cyano-4-phenyl-azetidin-3-yl)-acetic acid ethyl ester 24. Yield: 36%; mp: 68–70 °C; R_f: 0.5 (E–CyH, 1 : 1); $[\alpha]_D^{25}$ −68.7 (*c* 1.5, $CHCl_3$); δ_H(250 MHz, $CDCl_3$, Me_4Si): 1.10 (t, 3H, $J = 6.8$, CH_3), 2.48 (ABX syst., 1H, CHHCO_2Et), 2.50 (ABX syst., 1H, CHHCO_2Et), 2.83 (dquad, 1H, $J = 5.9$ and 6.0, $CHCH_2$), 3.49 (d, 1H, $J = 7.7$, CHPh), 3.52 (d, $J =$ 12.9, CHHPh), 3.75 (d, 1H, $J = 7.1$, CHCN), 3.83 (d, $J = 12.9$, CHHPh), 4.00 (dquad, 2H, $J = 1.5$ and 7.4, CH_2O), 7.18–7.38 (m, 10H, Ar); δ_C(62.9 MHz, $CDCl_3$, Me_4Si): 14.2 (CH_3), 36.4 (CH_2), 43.6, 53.1 (CH), 59.9, 61.2 (CH_2), 71.3 (CH), 118.9 (CN), 127.1, 128.0, 128.5, 128.6, 128.7, 129.6 (CHAr), 135.0, 139.8 (CqAr), 170.3 (CO); Anal. Calcd. for $C_{21}H_{22}N_2O_2$: C, 75.42; H, 6.63; N, 8.38. Found: C, 75.27; H, 6.72; N, 8.12%.

(3*R*,4*S*,5*S*)-(1-Benzyl-2-cyano-4-methyl-azetidin-3-yl)-acetic acid ethyl ester 25. R = Me: overall yield: 74%. *Cis–trans* ratio, 37 : 63.

Yield: 27%; R_f: 0.59 (E–CyH, 1 : 1); $[\alpha]_D^{25}$ +101.5 (*c* 0.2, $CHCl_3$); IR (neat): 2966, 2925, 2848, 2223, 1726 cm^{-1}; δ_H(250 MHz, $CDCl_3$, Me_4Si): 1.05 (d, 3H, $J = 6.2$, CH_3), 1.18 (t, 3H, $J =$ 7.1, CH_3), 2.54–2.70 (m, 3H, $CHCH_2CO_2Et$), 3.10–3.23 (m, 1H, $CHCH_3$), 3.62 (d, 1H, $J = 13.3$, CHHPh), 3.75 (d, 1H, $J = 13.3$, CHHPh), 4.06 (q, 2H, $J = 7.1$, CH_2O), 4.30 (d, 1H, $J = 5.9$, CHCN), 7.18–7.30 (m, 5H, Ar); δ_C(62.9 MHz, $CDCl_3$, Me_4Si): 14.3, 19.8 (CH_3), 34.5 (CH_2), 37.8, 55.9 (CH), 56.5, 61.0 (CH_2), 66.5 (CH), 116.1 (CN), 127.6, 128.6, 129.2 (CHAr), 136.9 (CqAr), 171.1 (CO); Anal. Calcd. for $C_{16}H_{20}N_2O_2$: C, 70.56; H, 7.40; N, 10.29. Found: C, 70.63; H, 7.58; N, 10.18%.

(3*S*,4*S*,5*S*)-(1-Benzyl-2-cyano-4-methyl-azetidin-3-yl)-acetic acid ethyl ester 26. Yield: 47%; R_f: 0.47 (E–CyH, 1 : 1); $[\alpha]_D^{25}$ −15.8 (*c* 0.5, $CHCl_3$); IR (neat): 2980, 2925, 2863, 2238, 1726, 1491, 1450 cm^{-1}; δ_H(250 MHz, $CDCl_3$, Me_4Si): 1.02 (d, 3H, $J =$ 5.9, CH_3), 1.19 (t, 3H, $J = 7.15$, CH_3), 2.42 (d, 1H, $J = 3.1$, CHHCO_2Et), 2.45 (d, 1H, $J = 0.9$, CHHCO_2Et), 2.52–2.67 (m, 1H, $CHCH_2$), 2.90 (qd, 1H, $J = 6$ and 7.5, 1H, $CHCH_3$), 3.35 (d, 1H, $J = 7.4$, CHCN), 3.64 (AB syst., 2H, $J = 12.9$, CH_2Ph), 4.08 (q, 2H, $J = 7.1$, CH_2O), 7.19–7.27 (m, 5H, Ar); δ_C(62.9 MHz, $CDCl_3$, Me_4Si): 14.3, 20.6 (CH_3), 36.7 (CH_2), 41.0, 54.3 (CH), 60.8, 61.2 (CH_2), 65.8 (CH), 119.0 (CN), 128.0, 128.6, 129.4 (CHAr), 135.7 (CqAr), 170.6 (CO); Anal. Calcd. for $C_{16}H_{20}N_2O_2$: C, 70.56; H, 7.40; N, 10.29. Found: C, 70.54; H, 7.51; N, 10.24%.

(3*R*,4*S*,5*S*)-(1-Benzyl-2-cyano-4-ethyl-azetidin-3-yl)-acetic acid ethyl ester 27. R = Et: overall yield: 73%. *Cis–trans* ratio, 42 : 58.

Yield: 31%; R_f: 0.69 (AcOEt–PE, 2 : 8); $[\alpha]_D^{25}$ +83 (*c* 0.6, $CHCl_3$); δ_H(300 MHz, $CDCl_3$, Me_4Si): 0.90 (t, 3H, $J = 7.5$, CH_3), 1.27 (t, 3H, $J = 7.5$, CH_3), 1.50–1.56 (m, 2H, CH_3CH_2), 2.64–2.84 (m, 3H, $CHCH_2CO_2Et$), 3.16 (q, 1H, $J = 6.8$, CHN), 3.74 (d, 1H, $J = 13.2$, CHHPh), 3.87 (d, 1H, $J = 13.2$, CHHPh), 4.18 (q, 2H, $J = 7.1$, CH_2O), 4.38 (d, 1H, $J = 7.4$, CHCN), 7.27–7.31 (m, 5H, Ar); δ_C(62.9 MHz, $CDCl_3$, Me_4Si): 9.3, 14.1 (CH_3), 27.0, 35.0 (CH_2), 35.3, 55.6 (CH), 56.8, 60.9 (CH_2), 71.6 (CH), 117.0 (CN), 127.4, 128.4, 128.9 (CHAr), 138.1 (CqAr), 171.4 (C=O);

MS (CI, NH_3): *m/z* 286.6, 91.9; Anal. Calcd. for $C_{17}H_{22}N_2O_2$: C, 71.30; H, 7.74; N, 9.78. Found: C, 71.23; H, 7.79; N, 9.74%.

(3*S*,4*R*,5*S*)-(1-Benzyl-2-cyano-4-ethyl-azetidin-3-yl)-acetic acid ethyl ester 28. Yield: 42%; R_f: 0.59 (AcOEt–PE, 2 : 8); $[\alpha]_D^{25}$ −9 (*c* 0.8, $CHCl_3$); δ_H(300 MHz, $CDCl_3$, Me_4Si): 0.86 (t, 3H, *J* = 7.5, CH_3), 1.29 (t, 3H, *J* = 7.5, CH_3), 1.45–1.55 (m, 2H, CH_3CH_2), 2.44–261 (m, 2H, $CHCH_2CO_2Et$), 2.71–2.77 (m, 1H, $CHCH_2CO_2Et$), 2.87–2.93 (m, 1H, CHN), 3.45 (d, 1H, *J* = 7.5, CHCN), 3.66 (d, 1H, *J* = 12.9, CHHPh), 3.81 (d, 1H, *J* = 12.9, CHHPh), 4.16 (q, 2H, *J* = 7.3, CH_2O), 7.25–7.31 (m, 5H, Ar); δ_C(62.9 MHz, $CDCl_3$, Me_4Si): 9.2, 14.2 (CH_3), 27.8, 37.4 (CH_2), 38.5, 54.0 (CH), 61.0, 61.4 (CH_2), 71.4 (CH), 119.1 (CN), 127.8, 128.5, 129.2 (CHAr), 136.1 (CqAr), 170.5 (CO); MS (CI, NH_3): *m/z* 286.7, 91.9; Anal. Calcd. for $C_{17}H_{22}N_2O_2$: C, 71.30; H, 7.74; N, 9.78. Found: C, 71.31; H, 7.83; N, 9.75%.

(3*R*,4*S*,5*S*)-(1-Benzyl-2-cyano-4-propyl-azetidin-3-yl)-acetic acid ethyl ester 29. R = Pr: overall yield: 72%. *Cis–trans* ratio, 37 : 63.

Yield: 27%; R_f: 0.72 (AcOEt–PE, 2 : 8); $[\alpha]_D^{25}$ +75 (*c* 0.8, $CHCl_3$); δ_H(300 MHz, $CDCl_3$, Me_4Si): 0.92 (t, 3H, *J* = 7.5, CH_3), 1.32 (t, 3H, *J* = 7.5, CH_3), 1.40–1.58 (m, 4H, CH_3 (CH_2)$_2$), 2.62–2.86 (m, 3H, $CHCH_2CO_2Et$), 3.10 (q, 1H, *J* = 6.8, CHN), 3.65 (d, 1H, *J* = 13.2, CHHPh), 3.76 (d, 1H, *J* = 13.2, CHHPh), 4.18 (q, 2H, *J* = 7.1, CH_2O), 4.28 (d, 1H, *J* = 6.3, CHCN), 7.27–7.32 (m, 5H, Ar); δ_C(62.9 MHz, $CDCl_3$, Me_4Si): 14.1, 14.3 (CH_3), 18.5, 34.5 (CH_2), 35.9, (CH), 36.7 (CH_2), 55.6 (CH), 56.8, 60.6 (CH_2), 70.1 (CH), 116.0 (CN), 127.5, 128.4, 128.9 (CHAr), 136.8 (CqAr), 170.9 (C=O); MS (CI, NH_3): *m/z* 300.6, 91.9.

(3*S*,4*R*,5*S*)-(1-Benzyl-2-cyano-4-propyl-azetidin-3-yl)-acetic acid ethyl ester 30. Yield: 45%; R_f: 0.62 (AcOEt–PE, 2 : 8); $[\alpha]_D^{25}$ −18 (*c* 0.7, $CHCl_3$); δ_H(300 MHz, $CDCl_3$, Me_4Si): 0.88 (t, 3H, *J* = 7.5, CH_3), 1.29 (t, 3H, *J* = 7.5, CH_3), 1.32–1.56 (m, 4H, CH_3 (CH_2)$_2$), 2.48–2.65 (m, 2H, $CHCH_2CO_2Et$), 2.75–2.79 (m, 1H, $CHCH_2CO_2Et$), 2.85–2.99 (m, 1H, CHN), 3.45 (d, 1H, *J* = 7.5, CHCN), 3.66 (d, 1H, *J* = 12.9, CHHPh), 3.85 (d, 1H, *J* = 12.9, CHHPh), 4.15 (q, 2H, *J* = 7.3, CH_2O), 7.25–7.31 (m, 5H, Ar); δ_C(62.9 MHz, $CDCl_3$, Me_4Si): 14.1 ($2CH_3$), 18.3, 37.3, 37.5 (CH_2), 39.1, 54.0 (CH), 61.0, 61.4 (CH_2), 69.5 (CH), 114.0 (CN), 127.4, 128.5, 128.9 (CHAr), 135.8 (CqAr), 170.5 (CO).

(3*R*,4*S*,5*S*)-(1-Benzyl-2-cyano-4-isopropyl-azetidin-3-yl)-acetic acid ethyl ester 31. R = *i*-Pr: overall yield: 87%. *Cis–trans* ratio, 30 : 70.

Yield: 26%; R_f: 0.67 (AcOEt–PE, 2 : 8); $[\alpha]_D^{25}$ +81 (*c* 1.1, $CHCl_3$); δ_H(300 MHz, $CDCl_3$, Me_4Si): 0.73 and 0.74 (2d, 6H, *J* = 6.8, CH_3), 1.05 (t, 3H, *J* = 7.1, CH_3), 1.53–1.61 (m, 1H, $(CH_3)_2CH$), 2.41–2.66 (m, 2H, $CHCH_2CO_2Et$), 2.57–2.58 (m, 1H, $CHCH_2CO_2Et$), 2.81–2.84 (m, 1H, CHN), 3.54 (d, 1H, *J* = 13.5, CHHPh), 3.72 (d, 1H, *J* = 13.5, CHHPh), 3.93 (q, 2H, *J* = 7.1, CH_2O), 4.13 (d, 1H, *J* = 7.4, CHCN), 7.06–7.13 (m, 5H, Ar); δ_C(62.9 MHz, $CDCl_3$, Me_4Si): 14.1, 17.7, 19.0 (CH_3), 31.8, 32.7 (CH), 35.5 (CH_2), 55.3 (CH), 57.2, 60.9 (CH_2), 75.4 (CH), 116.0 (CN), 127.4, 128.4, 128.9 (CHAr), 136.9 (CqAr), 170.9 (C=O); MS (CI, NH_3): *m/z* 286.6, 91.9; Anal. Calcd. for $C_{18}H_{24}N_2O_2$: C, 71.97; H, 8.05; N, 9.33. Found: C, 71.84; H, 8.14; N, 9.24%.

(3*S*,4*R*,5*S*)-(1-Benzyl-2-cyano-4-isopropyl-azetidin-3-yl)-acetic acid ethyl ester 32. Yield: 61%; R_f: 0.52 (AcOEt–PE, 2 : 8); $[\alpha]_D^{25}$ +1.5 (*c* 1.5, $CHCl_3$); δ_H(300 MHz, $CDCl_3$, Me_4Si): 0.74 and 0.75 (2d, 6H, *J* = 6.9, CH_3), 1.12 (t, 3H, *J* = 7.1, CH_3), 1.53–1.64 (m, 1H, $(CH_3)_2CH$), 2.25–2.62 (m, 4H, $CHCH_2CO_2Et$ and CHN), 3.27 (d, 1H, *J* = 7.1, CHCN), 3.42 (d, 1H, *J* = 13.0, CHHPh), 3.77 (d, 1H, *J* = 13.0, CHHPh), 4.00 (q, 2H, *J* = 7.1, CH_2O), 7.12–7.25 (m, 5H, Ar); δ_C(62.9 MHz, $CDCl_3$, Me_4Si): 14.1, 17.6, 18.7 (CH_3), 32.8, 36.2 (CH), 38.0 (CH_2), 53.8 (CH), 61.0, 62.2 (CH_2), 75.1 (CH), 119.2 (CN), 127.8, 128.5, 129.1 (CHAr), 136.0 (CqAr), 170.5 (CO); MS (CI, NH_3): *m/z* 286.6, 91.9; Anal. Calcd. for $C_{18}H_{24}N_2O_2$: C, 71.97; H, 8.05; N, 9.33. Found: C, 71.87; H, 8.15; N, 9.25%.

(3*R*,4*S*,5*S*)-(1-Benzyl-2-cyano-4-isobutyl-azetidin-3-yl)-acetic acid ethyl ester 33. R = *i*-Bu: overall yield: 86%. *Cis–trans* ratio, 27 : 63.

Yield: 32%; R_f: 0.77 (AcOEt–PE, 2 : 8); $[\alpha]_D^{25}$ +85 (*c* 1.2, $CHCl_3$); δ_H(300 MHz, $CDCl_3$, Me_4Si): 0.88 and 0.91 (2d, 6H, *J* = 5.1, CH_3), 1.25 (t, 3H, *J* = 7.0, CH_3), 1.41–1.62 (m, 3H, $(CH_3)_2CHCH_2$), 2.61–2.83 (m, 3H, $CHCH_2CO_2Et$), 3.24 (q, 1H, *J* = 6.4, CHN), 3.56 (d, 1H, *J* = 12.6, CHHPh), 3.80 (d, 1H, *J* = 12.6, CHHPh), 4.13 (q, 2H, *J* = 7.1, CH_2O), 4.35 (d, 1H, *J* = 6.1, CHCN), 7.28–7.35 (m, 5H, Ar); δ_C(62.9 MHz, $CDCl_3$, Me_4Si): 14.1, 22.8, 23.2 (CH_3), 24.8 (CH), 34.8 (CH_2), 36.7 (CH), 44.5, 55.6 (CH_2), 56.8 (CH), 60.8 (CH_2), 68.5 (CH), 116.0 (CN), 127.4, 128.4, 128.9 (CHAr), 136.7 (CqAr), 170.8 (CO); MS (CI, NH_3): *m/z* 315.3, 314.4, 92.4; Anal. Calcd. for $C_{19}H_{26}N_2O_2$: C, 72.58; H, 8.33; N, 8.91. Found: C, 72.39; H, 8.44; N, 8.86%.

(3*S*,4*R*,5*S*)-(1-Benzyl-2-cyano-4-isobutyl-azetidin-3-yl)-acetic acid ethyl ester 34. Yield: 54%; R_f: 0.53 (AcOEt–PE, 2 : 8); $[\alpha]_D^{25}$ +5 (*c* 1.5, $CHCl_3$); δ_H(300 MHz, $CDCl_3$, Me_4Si): 0.85 and 0.88 (2d, 6H, *J* = 6.0, CH_3), 1.28 (t, 3H, *J* = 7.1, CH_3), 1.38–170 (m, 3H, $(CH_3)_2CHCH_2$), 2.40–2.77 (m, 3H, $CHCH_2CO_2Et$), 3.02 (q, 1H, *J* = 6.6, CHN), 3.44 (d, 1H, *J* = 7.5, CHCN), 3.53 (d, 1H, *J* = 13.0, CHHPh), 3.69 (d, 1H, *J* = 13.0, CHHPh), 4.16 (q, 2H, *J* = 7.1, CH_2O), 7.25–7.45 (m, 5H, Ar); δ_C(62.9 MHz, $CDCl_3$, Me_4Si): 14.1, 22.8, 23.1 (CH_3), 24.5 (CH), 37.2 (CH_2), 39.8 (CH), 45.1, 54.0 (CH_2), 61.0, 61.4, 67.9 (CH), 119.0 (CN), 127.9, 128.5, 129.2 (CHAr), 135.7 (CqAr), 170.4 (CO); MS (CI, NH_3): *m/z* 315.3, 314.4, 92.3; Anal. Calcd. for $C_{19}H_{26}N_2O_2$: C, 72.58; H, 8.33; N, 8.91. Found: C, 72.73; H, 8.49; N, 8.78%.

General procedure for the hydrolysis–debenzylation steps

An emulsion of 2-cyano azetidine (0.75 mmol) in a 1 : 1 mixture of CCl_4 and 6N aqueous HCl (20 mL) was heated for 48 h with vigorous stirring at 80 °C. The mixture was cooled to rt and concentrated to dryness under reduced pressure. The crude residue was washed with small portions of diethyl ether to give the crude chlorhydrate that was used as such for the next step.

A suspension of the above hydrochloride (0.484 mmol) in ethanol (15 mL) was stirred until complete dissolution. Palladium on charcoal (10% wt., 60 mg) was then added and the suspension was vigorously stirred under an atmosphere of hydrogen (balloon) for 4 h. After filtration on Celite, the filtrate was evaporated to dryness. The residue was dissolved in the minor amount of water and this solution was deposited on an ion exchange resin (Dowex 50 × 8, H^+ form, 3 g) in a chromatography column. The resin was washed with water until neutrality and the amino acid was then eluted with an aqueous solution of ammonia (1%). The elution of the TLCs was done with EtOH–40% aqueous NH_3–H_2O = 9 : 3 : 1. Positive fractions were then lyophilized to give amino acids as solids.

(2*R*,3*S*,4*S*)-3-Carboxymethyl-4-methyl-azetidine-2-carboxylic acid 35. Yield: 80%; R_f = 0.29 (EtOH–40%NH_4OH–H_2O, 9 : 3 : 1); $[\alpha]_D^{25}$ +97 (*c* 0.2, H_2O); δ_H(200 MHz, D_2O, Me_4Si): 1.56 (d, 3H, *J* = 6.6, Me), 2.26 (dd, 1H, *J* = 11.4, 15.4, CHHCOOH), 2.50 (dd, 1H, *J* = 4.8, 15.4, CHHCOOH), 3.06 (dddd, 1H, *J* = 4.8, 7.5, 10.1, 11.9, $CHCH_2$), 4.19 (quint, 1H, *J* = 7.0, CHMe), 4.70 (d, 1H, *J* = 10.1, NCHCOOH); δ_C(75.5 MHz, D_2O, Me_4Si): 21.3 (CH_3), 43.0 (CH_2), 45.3, 62.4, 63.0 (CH), 176.3, 181.5 (CO); Anal. Calcd. for $C_7H_{11}NO_4$, 0.75 NH_3, 1.50 H_2O: C, 39.48; H, 7.69; N, 11.51. Found: C, 39.33; H, 8.02; N, 11.51%; MS (CI, CH_4): *m/z* 174 (MH^+, 19), 173 (34), 156 (100), 143 (10), 130 (12), 128 (19); HRMS (CI, CH_4): MH^+ ($C_7H_{12}NO_4$) Calcd. 174.0766, Found 174.0769.

(2*S*,3*S*,4*S*)-3-Carboxymethyl-4-methyl-azetidine-2-carboxylic acid 36. Yield: 66%; R_f = 0.25 (EtOH–40%NH_4OH–H_2O,

9 : 3 : 1); $[\alpha]_D^{25}$ +9.5 (*c* 0.2, H_2O); δ_H(200 MHz, D_2O, Me_4Si): 1.51 (d, 3H, *J* = 7.0, Me), 2.38–2.91 (m, 3H, C*H*–CH_2), 4.21 (quint, 1H, *J* = 8.8, C*H*Me), 4.33 (d, 1H, *J* = 8.6, NC*H*COOH); δ_C(75.5 MHz, D_2O, Me_4Si): 21.3 (CH_3), 43.0 (CH_2), 45.3 (CH), 62.4 (CH), 63.0 (CH), 176.3, 181.5 (CO); Anal. Calcd. for $C_7H_{11}NO_4$, 0.75 NH_3, 1 H_2O: C, 41.22; H, 7.54; N, 12.02. Found: C, 41.40; H, 8.13; N, 12.03%; MS (CI, CH_4): *m*/*z* 174 (MH^+, 22), 173 (55), 156 (100), 130 (26), 128 (35); HRMS (CI, CH_4): MH^+ ($C_7H_{12}NO_4$) Calcd. 174.0766, Found 174.0764.

(2*R*,3*S*,4*S*)-3-Carboxymethyl-4-ethyl-azetidine-2-carboxylic acid 37. Yield: 71%; R_f = 0.85 (EtOH–40%NH_4OH–H_2O, 9 : 3 : 1); $[\alpha]_D^{25}$ +140 (*c* 0.2, H_2O); δ_H(300 MHz, D_2O, Me_4Si): 0.83 (t, 3H, *J* = 7.5, Me), 1.84 (quint, 2H, *J* = 7.5, CH_2CH$_3$), 2.14–2.45 (m, 2H, CH_2COOH), 2.97–3.08 (m, 1H, C*H*CH$_2$COOH), 3.92 (q, 1H, *J* = 7.3, C*H*N), 4.60 (d, 1H, *J* = 10.2, NC*H*COOH); δ_C(75.5 MHz, D_2O, Me_4Si): 8.6 (CH_3), 25.7, 37.3 (CH_2), 37.7, 59.1, 66.6 (CH), 171.5, 178.7 (CO); MS (CI, NH_3): *m*/*z* 176.1, 165.0, 158.0, 140.0, 115.0, 112.0, 96.0; EIHRMS: M^+ ($C_8H_{13}NO_4$) Calcd. 187.084, Found 187.087.

(2*S*,3*S*,4*S*)-3-Carboxymethyl-4-ethyl-azetidine-2-carboxylic acid 38. Yield: 58%; R_f = 0.88 (EtOH–40%NH_4OH–H_2O, 9 : 3 : 1); $[\alpha]_D^{25}$ 0 (*c* 0.3, H_2O); δ_H(300 MHz, D_2O, Me_4Si): 0.83 (t, 3H, *J* = 7.5, Me), 1.78 (quint, 2H, *J* = 7.5, CH_2CH$_3$), 2.35–2.51 (m, 2H, CH_2COOH), 2.72–2.79 (m, 1H, C*H*CH$_2$COOH), 3.96 (q, 1H, *J* = 7.5, C*H*N), 4.28 (d, 1H, *J* = 8.3, NC*H*COOH); δ_C(75.5 MHz, D_2O, Me_4Si): 8.5 (CH_3), 25.9, 40.5 (CH), 40.6 (CH_2), 60.3, 64.8 (CH), 173.5, 178.9 (CO); MS (CI, NH_3): *m*/*z* 199.1, 186.1, 168.1, 140.1, 112.1, 96.0, 78.0, 63.0; EIHRMS: M^+ ($C_8H_{13}NO_4$) Calcd. 187.084, Found 187.087.

(2*R*,3*S*,4*S*)-3-Carboxymethyl-4-propyl-azetidine-2-carboxylic acid 39. Yield: 73%; R_f = 0.85 (EtOH–40%NH_4OH–H_2O, 9 : 3 : 1); $[\alpha]_D^{25}$ +105 (*c* 0.2, H_2O); δ_H(300 MHz, D_2O, Me_4Si): 0.89 (t, 3H, *J* = 7.5, Me), 1.22–1.50 (m, 2H, CH_2CH$_3$), 1.75–1.85 (m, 2H, CH_2CH$_2$CH$_3$), 2.21 (dd, 1H, *J* = 8, 15, CH*H*COOH), 2.51 (dd, 1H, *J* = 4, 15, C*H*HCOOH), 3.01–3.18 (m, 1H, C*H*CH$_2$COOH), 4.12 (q, 1H, *J* = 7.3, C*H*N), 4.75 (d, 1H, *J* = 10.2, NC*H*COOH); EIHRMS: M^+ ($C_9H_{15}NO_4$) Calcd. 201.100, Found 201.099.

(2*S*,3*S*,4*S*)-3-Carboxymethyl-4-propyl-azetidine-2-carboxylic acid 40. Yield: 68%; R_f = 0.82 (EtOH–40%NH_4OH–H_2O, 9 : 3 : 1); $[\alpha]_D^{25}$ +5 (*c* 0.2, H_2O); δ_H(300 MHz, D_2O, Me_4Si): 0.88 (t, 3H, *J* = 7.5, Me), 1.20–1.52 (m, 2H, CH_2CH$_3$), 1.75–1.95 (m, 2H, CH_2CH$_2$CH$_3$), 2.60–3.01 (m, 3H, C*H*CH_2COOH), 4.18 (q, 1H, *J* = 7.3, C*H*N), 4.55 (d, 1H, *J* = 10.5, NC*H*COOH); EIHRMS: M^+ ($C_9H_{15}NO_4$) Calcd. 201.100, Found 201.099.

(2*R*,3*S*,4*S*)-3-Carboxymethyl-4-isopropyl-azetidine-2-carboxylic acid 41. Yield: 49%; R_f = 0.87 (EtOH–40%NH_4OH–H_2O, 9 : 3 : 1); mp: 152 °C; $[\alpha]_D^{25}$ +32 (*c* 0.3, H_2O); δ_H(300 MHz, D_2O, Me_4Si): 0.83 (d, 3H, *J* = 6.5, Me), 0.89 (d, 3H, *J* = 6.5, Me), 1.99–2.11 (m, 1H, C*H*(Me)$_2$), 2.21–2.48 (m, 2H, CH_2COOH), 3.08–3.19 (m, 1H, C*H*CH$_2$COOH), 3.71 (t, 1H, *J* = 7.5, C*H*N), 4.55 (d, 1H, *J* = 10.2, NC*H*COOH); δ_C(75.5 MHz, D_2O, Me_4Si): 16.3, 17.2 (CH_3), 30.8, 35.9 (CH), 37.8 (CH_2), 56.4, 70.3 (CH), 171.4, 178.0 (CO); MS (CI, NH_3): *m*/*z* 199.1, 183.1, 156.1, 140.0, 122.1, 112.0, 91.0, 78.0; EIHRMS: M^+ ($C_9H_{15}NO_4$) Calcd. 201.100, Found 201.096.

(2*S*,3*S*,4*S*)-3-Carboxymethyl-4-isopropyl-azetidine-2-carboxylic acid 42. Yield: 43%; R_f = 0.81 (EtOH–40%NH_4OH–H_2O, 9 : 3 : 1); mp: 113 °C; $[\alpha]_D^{25}$ 0 (*c* 0.3, H_2O); δ_H(300 MHz, D_2O, Me_4Si): 0.56 (d, 3H, *J* = 6.5, Me), 0.62 (d, 3H, *J* = 6.5, Me), 1.65–1.77 (m, 1H, C*H*(Me)$_2$), 2.14–2.31 (m, 2H, CH_2COOH), 2.51–2.63 (m, 1H, C*H*CH$_2$COOH), 3.51 (t, 1H, *J* = 7.0, C*H*N), 4.05 (d, 1H, *J* = 10.2, NC*H*COOH); δ_C(75.5 MHz, D_2O, Me_4Si): 16.0, 17.0 (CH_3), 30.8, 39.6 (CH), 40.8 (CH_2), 59.5, 68.5 (CH), 173.3, 178.7 (CO); MS (CI, NH_3): *m*/*z* 202.1, 79.0, 64.0; EIHRMS: M^+ ($C_9H_{15}NO_4$) Calcd. 201.100, Found 201.096.

(2*R*,3*S*,4*S*)-3-Carboxymethyl-4-isobutyl-azetidine-2-carboxylic acid 43. Yield: 64%; R_f = 0.79 (EtOH–40%NH_4OH–H_2O, 9 : 3 : 1); mp: 185 °C; $[\alpha]_D^{25}$ +46 (*c* 0.3, H_2O); δ_H(300 MHz, D_2O, Me_4Si): 0.57 (d, 6H, *J* = 6.5, 2Me), 1.23–1.65 (m, 3H, CH_2C*H*Me$_2$), 1.96–2.23 (m, 2H, CH_2COOH), 2.75–2.84 (m, 1H, C*H*CH$_2$COOH), 3.80–3.98 (m, 1H, C*H*N), 4.37 (d, 1H, *J* = 8.9, NC*H*COOH); δ_C(75.5 MHz, D_2O, Me_4Si): 21.0, 21.8 (CH_3), 24.9 (CH), 37.1 (CH_2), 37.3 (CH), 41.5 (CH_2), 59.3, 63.6 (CH), 171.4, 178.0 (CO); MS (CI, NH_3): *m*/*z* 237.1, 213.0, 199.0, 176.1, 153.0, 142.1, 126.1, 112.1; EIHRMS: M^+ ($C_{10}H_{17}NO_4$) Calcd. 215.116, Found 215.116.

(2*S*,3*S*,4*S*)-3-Carboxymethyl-4-isobutyl-azetidine-2-carboxylic acid 44. Yield: 77%; R_f = 0.88 (EtOH–40%NH_4OH–H_2O, 9 : 3 : 1); mp: 192 °C; $[\alpha]_D^{25}$ 0 (*c* 0.3, H_2O); δ_H(300 MHz, D_2O, Me_4Si): 0.81 (d, 6H, *J* = 6.5, 2Me), 1.59–1.76 (m, 3H, CH_2C*H*Me$_2$), 2.43–2.63 (m, 2H, CH_2COOH), 2.73–2.77 (m, 1H, C*H*CH$_2$COOH), 4.10–4.14 (m, 1H, C*H*N), 4.31 (d, 1H, *J* = 8.4, NC*H*COOH); δ_C(75.5 MHz, D_2O, Me_4Si): 21.1, 21.8 (CH_3), 23.9 (CH), 39.8 (CH_2), 41.4 (CH), 41.7 (CH_2), 60.5, 61.8 (CH), 173.4, 178.3 (CO); MS (CI, NH_3): *m*/*z* 244.2, 216.2, 157.0, 139.0, 93.0, 81.0, 78.0; EIHRMS: M^+ ($C_{10}H_{17}NO_4$) Calcd. 215.116, Found 215.116.

(4*S*)-[Benzyl-(2-hydroxy-1-methylethyl)-amino]-acetic acid *tert*-butyl ester 45. To a solution of amino alcohol **6** (2.5 g, 15.2 mmol) in DMF (65 mL) were added sodium iodide (4.5 g, 30.5 mmol), potassium hydrogencarbonate (3.05 g, 30.5 mmol) and *tert*-butylbromoacetate (5.9 g, 30.5 mmol). After stirring at rt for 12 h, the mixture was treated with water (100 mL). Extraction with DCM and the usual workup gave amino alcohol **45** after purification (E–CyH = 1 : 1) by flash chromatography (3.22 g, 76%).

*R*f = 0.38 (E–CyH = 6 : 4); $[\alpha]_D^{25}$ +34.0 (*c* 1.3, $CHCl_3$); IR (film): 3868, 3462, 2976, 2930, 1726, 1455, 1363 cm^{-1}; δ_H(250 MHz, $CDCl_3$, Me_4Si): 1.01 (d, 3H, *J* = 6.8, CH_3), 1.45 (s, 9H, *t*-Bu), 2.99–3.13 (m, 1H, C*H*Me), 3.13 (d, 1H, *J* = 17.0, C*H*HPh), 3.29 (d, 1H, *J* = 17.3, CH*H*Ph), 3.34–3.55 (m, 2H, CH_2OH), 3.67 (d, 1H, *J* = 13.6, NC*H*H), 3.90 (d, 1H, *J* = 13.6, NCH*H*), 3.99 (broadd, 1H, *J* = 7.1, OH), 7.25–7.45 (m, 5H, Ph); δ_C(62.9 MHz, $CDCl_3$, Me_4Si): 10.3, 28.2 (CH_3), 51.4, 55.1 (CH_2), 57.2 (CH), 63.6 (CH_2) 81.3 (Cq), 127.3, 128.4, 128.9 (CHAr), 138.9 (CqAr), 172.0 (CO); Anal. Calcd. for $C_{16}H_{25}NO_3$: C, 68.79; H, 9.02; N, 5.01. Found: C, 68.54; H, 9.16; N, 4.91%.

(2*E*,4*S*)-4-(Benzyl-*tert*-butoxycarbonylmethyl-amino)-pent-2-enoic acid ethyl ester 46. Following the general procedure for the synthesis of **17–22**, and starting from **45**, compound **46** was obtained as an oil.

Yield: 77%; R_f = 0.58 (E–CyH = 6 : 4); $[\alpha]_D^{25}$ −31.8 (*c* 0.9, $CHCl_3$); IR (film): 2976, 2925, 1726, 1644, 1454 cm^{-1}; δ_H(250 MHz, $CDCl_3$, Me_4Si): 1.11 (d, 3H, *J* = 6.8, Me), 1.19 (t, 3H, *J* = 7.1, Me), 1.34 (s, 9H, *t*-Bu), 3.12 (AB syst., 2H, *J* = 17.0, CH_2Ph), 3.54 (dquint, 1H, *J* = 1.3, 6.8, C*H*Me), 3.70 (s, 2H, NCH_2CO), 4.11 (quad, 2H, *J* = 7.3, CH_2CH$_3$), 5.85 (dd, 1H, *J* = 1.2, 15.7, C*H*CO$_2$Et), 6.84 (dd, 1H, *J* = 6.5, 15.7, C*H*=CH), 7.08–7.31 (m, 5H, Ph); δ_C(62.9 MHz, $CDCl_3$, Me_4Si): 14.3, 16.3, 28.2 (Me), 51.8, 55.2 (CH_2), 56.3 (CH), 60.4 (CH_2), 80.9 (Cq), 121.9 (CH), 128.1, 128.4, 128.7 (CHAr), 139.4 (CqAr), 150.4 (CH), 166.5, 171.2 (CO); Anal. Calcd. for $C_{20}H_{29}NO_4$: C, 68.14; H, 8.41; N, 4.03. Found: C, 69.21; H, 8.59; N, 4.00%.

(2*S*,3*S*,4*S*)-1-Benzyl-3-ethoxycarbonylmethyl-4-methyl-azetidine-2-carboxylic acid *tert*-butyl ester 47. To a solution of **45** (0.35 mmol, 121 mg) in THF (6 mL) was added, at −78 °C, a 1 M solution of potassium *tert*-butylate in THF (0.35 mL, 0.35 mmol). The mixture was warmed to −50 °C within one hour and was hydrolyzed by addition of a saturated aqueous solution of NH_4Cl. Usual workup was followed by purification by preparative TLC (E–CyH, 6 : 4), which gave **47** as an oil (60 mg, 50%). R_f = 0.52 (E–CyH = 60 : 40);

δ_H(200 MHz, $CDCl_3$, Me_4Si): 1.03 (d, 3H, J = 6.2, Me), 1.25 (t, 3H, J = 7.0, Me), 1.38 (s, 9H, *t*-Bu), 2.35–2.55 (m, 3H, C*H*CH_2CO$_2$Et), 2.84 (quint, 1H, J = 7.0, C*H*Me), 3.17 (d, 1H, J = 7.5, NC*H*CO), 3.62 (d, 1H, J = 12.7, C*H*HPh), 3.79 (d, 1H, J = 12.7, CH*H*Ph), 4.10 (quad, 2H, J = 7.0, CH_2CH$_3$), 7.18–7.36 (m, 10H, Ph); δ_C(75.5 MHz, $CDCl_3$, Me_4Si): 14.3, 20.6, 28.0 (CH_3), 37.6 (CH_2), 39.5 (CH), 60.5, 61.4 (CH_2), 64.7, 68.1 (CH), 80.8 (Cq), 127.1, 128.1, 129.6 (CHAr), 137.4 (CqAr), 171.2, 171.4 (CO).

Molecular modeling study of azetidine analogues 36, 38, 40, 42, 44 and other selected ligands

The modeling study was performed using the software package MOE (Molecular Operating Environment, v2004.03, Chemical Computing Group, 2004) using the built-in mmff94 force field and the GB/SA continuum solvent model. Each compound was submitted to a stochastic conformational search and with respect to its global minimum returned (ΔG in kcal mol^{-1}), conformations above +7 kcal mol^{-1} were discarded. For all azetidine analogues, the γ-carboxylate group was protonated prior to execution of the conformational search, as this gave a larger and thus more reliable number of output conformations. Systematic rotation of the carboxymethyl group was carried out in steps of 10/360° for the C(sp^3)–C(sp^3) bond and in steps of 60/360° for the C(sp^3)–C(sp^2) bond and each conformation energy minimized. Systematic rotation of the R group was carried out in steps of 10/360° of the C(sp^3)–C(sp^3) bond and each conformation energy minimized. Superimpositions of amino acid ligands were carried out using the built-in function in MOE, by fitting the ammonium group and the two carboxylate groups.

Pharmacological characterization at iGluR and mGluR. Rat brain membrane preparations used in the receptor binding experiments were prepared according to the method described by Ransom and Stec.[26] Affinity for AMPA,[27] KAIN,[28] and NMDA[29] receptor sites was determined using 5 nM [^{3}H]AMPA, 5 nM [^{3}H]KAIN, and 2 nM [^{3}H]CGP 39653 with some modifications previously described.[30]

Pharmacological characterization at human EAAT1–3. The pharmacological properties of azetidine analogues at human EAAT1, EAAT2 and EAAT3 were determined in the FLIPR® Membrane Potential (FMP) assay. The construction of human embryonic kidney 293 (HEK293) cell lines stably expressing human EAAT1, EAAT2 and EAAT3 have been reported previously, and the pharmacological characterization of the C-4-alkyl analoges of *t*-CAA were performed essentially as described here.[31] Briefly, cells were split into poly-D-lysine-coated black walled clear-bottom 96-well plates in Dulbecco's Modified Eagle Medium supplemented with penicillin (100 U ml^{-1}), streptomycin (100 μg ml^{-1}), 10% dialysed fetal bovine serum and 1 mg ml^{-1} G-418. The medium was aspirated 16–24 h later, and the cells were washed with 100 μL Krebs buffer [140 mM NaCl–4.7 mM KCl–2.5 mM $CaCl_2$–1.2 mM $MgCl_2$–11 mM HEPES–10 mM D-glucose, pH 7.4]. Then 50 μL Krebs buffer were added to each well (in the characterization of non-substrate inhibitors, the inhibitors were added to this buffer). 50 μL Krebs buffer supplemented with FMP assay dye were then added to each well, and the plate was incubated at 37 °C for 30 min. The plate was assayed at room temperature in a NOVOstar™ plate reader measuring emission at 560 nm caused by excitation at 530 nm before and up to 1 min after addition of 25 μL substrate solution. The experiments were performed in duplicate at least three times for each compound. For the characterization of non-substrate inhibitors, 30 μM Glu were used as substrate concentration. Concentration–response curves were constructed based on the maximal responses obtained for 7 different concentrations of each compound. IC_{50} values were converted to K_i values by the use of the Cheng–Prusoff equation.[32]

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