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Rational Design, Synthesis and Pharmacological Evaluation of the (2*R*)- and (2*S*)-Stereoisomers of 3-(2-Carboxy-pyrrolidinyl)-2-methyl Acetic Acid as Ligands for the Ionotropic Glutamate Receptors

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In this paper we describe the rational design, synthesis and pharmacological evaluation of two new stereoisomeric (*S*)-glutamate (Glu) analogues. The rational design was based on hybrid structures of the natural product kainic acid, a synthetic analogue CPAA and the high-affinity Glu analogue SYM2081. Pharmacological evaluation of the two stereoisomers revealed that one stereoisomer showed a subtype selectivity profile with low micromolar affinity for GluK1 and GluK3 and a 10- to 15-fold lower affinity for GluK2. The other stereoisomer displayed full selectivity for the KA over AMPA and NMDA receptors (GluK1–3: 0.39, 0.51 and 0.099 μM, respectively).

Introduction

In the mammalian central nervous system (CNS), (*S*)-glutamate (Glu) functions as the major excitatory neurotransmitter. Once released from the pre-synaptic neuron into the glutamatergic synapse, Glu activates a number of pre- and post-synaptic glutamate receptors. On the basis of the pharmacological profile and ligand selectivity studies, the Glu receptors have been divided into two main classes: the fast acting ionotropic receptors (iGluRs),[1] and the G protein-coupled metabotropic receptors (mGluRs), which produce a much slower signal transduction through second messenger systems.[2] Uptake of Glu is mediated by action of the excitatory amino acid transporters (EAAT), previously termed the Glu transporters.[3] Furthermore, the iGluRs have been divided into three groups on the basis of ligand selectivity studies (new subunit nomenclature):[4] the α-amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA) receptors comprising subunits GluA1—4, the kainic acid (KA) receptors comprising subunits GluK1–5, and the *N*-methyl-D-aspartate (NMDA) receptors comprising subunits GluN1–3. Functional iGluRs are tetrameric in structure comprising four subunits with the ion channel pore formed in the center of the receptor.[5] For the KA receptors, subunits GluK1–3 may form functional homomeric[6] or heteromeric[7] receptors, whereas subunits GluK4 and GluK5[8] only form functional receptors in combination with subunits GluK1, GluK2 or GluK3. Although only one glutamate receptor-based drug is on the market (memantine for the treatment of Alzheimer's disease),[9] the glutamatergic neurotransmitter system is believed to hold great promise as it is involved in important neuro-physiological processes, such as memory and learning, motor function, and neural plasticity[10] and development.[11] Thus, psychiatric diseases or disorders such as depression,[12] anxiety,[13] addiction,[14] migraine,[15] and schizophrenia[16] may be directly related to disordered glutamatergic neurotransmission.[17] Moreover, excessive Glu signaling is neurotoxic and will result in neuronal death.[18] On this basis, it has been suggested that neurodegenerative diseases such as Alzheimer's, Huntington's, amyotrophic lateral sclerosis (ALS), cerebral stroke[16] and epilepsy[19] may be the result of a malfunctioning glutamatergic neurotransmitter system.

The usage of subtype-selective ligands is a valuable strategy to investigate the physiological role and function of a given Glu receptor subtype. However, despite an extensive effort, the discovery of such compounds has proven a major challenge as the orthosteric ligand binding pockets within an iGluR group display high homology. In summary for KA receptor agonists,[20] it has been shown that introduction of a substituent in the syn-2,4-position of Glu provides highly potent and selective homomeric GluK1 agonists.[21–23] Likewise, potent and selective GluK1 antagonists[24] have been discovered for the homomeric GluK1 subtype. In general, iGluR antagonism is achieved by increasing the distance between the α-amino acid moiety and the distal carboxylate group as exemplified by the recently published KA antagonist (2*S*,3*R*)-3-(3-carboxyphenyl)-pyrrolidine-2-carboxylic acid.[25] Herein, we describe the rational design and synthesis a new class of KA receptor ligands **1a**/**1b**, which are hybrid structures of reported KA ligands: KA, CPAA and SYM2081. Furthermore, the pharmacological profile

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of two C2 epimers **1a**/**1b** are investigated at native iGluR receptors and homomeric KA receptor subtypes GluK1–3.

Results and Discussion

For the Glu receptors, it has been shown from X-ray crystal structures that Glu binds to and activates the iGluRs in a folded conformation, whereas an extended form is observed as the binding mode when activating the class of mGluRs (Figure 1).[26] Furthermore, this observation has been supported by the pharmacological evaluation of a number of conformationally restricted analogues of Glu that either mimic the folded or the extended conformation.[26]

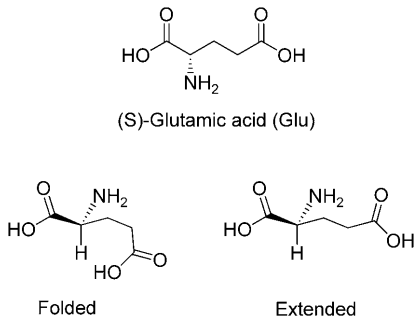
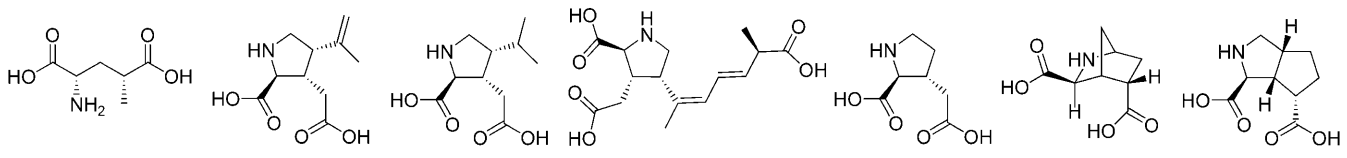


Figure 1. Chemical structure of (*S*)-glutamic acid (Glu) and illustration of the folded Glu-conformation and the extended Glu conformation.

However, it is noteworthy that an optimal low-energy conformation does not warrant high binding affinity as exemplified by the highly conformationally restricted Glu analogue DCAN (Table 1).[27] Even though this compound perfectly mimics the folded Glu conformation, it is without affinity for any of the iGluRs. In fact, the ability of a ligand to adopt an optimal binding conformation is only one parameter affecting its binding affinity. Steric clashes together with favorable interactions between a ligand and a receptor play essential roles, but also the ability of the protein to adopt a favorable conformation induced by the nature of the ligand.[28] Furthermore, the ability of the ligand to disrupt the organized water matrix in the receptor ligand binding domain (LBD) and exclude water molecules on domain closure adds positively to the overall binding (negative ΔG) by increasing the entropic term (ΔS).[29] Using static modeling techniques, the dynamics of the protein binding pocket, as well as the ΔS term of the water disruption process, cannot be calculated, rendering essential information outside of reach when attempting to estimate the binding affinity of a ligand in silico.[30]

KA, CPAA and SYM2081 (Table 1) were submitted to an in silico stochastic conformational search to determine their low-energy conformations. The calculated conformations all resembled the folded Glu conformation characterized as illustrated in Figure 1. Furthermore, the calculated low-energy conformations of KA and SYM2081 were similar to conformations observed when crystallized with the LBD (KA: GluA2, PDB: 1FTK,[31] and GluK2, PDB: 1TT1;[32] SYM2081: GluK2, PDB: 1SD3[32]). A three-point superimposition of calculated low-energy conformations of KA, CPAA and SYM2081 (Figure 2a) clearly shows that their conformations are highly similar and perfectly mimic the folded Glu conformation. However, on comparison with binding affinity data (Table 1) the three compounds are indeed distinct. Whereas KA displays 7 nM binding affinity for native KA receptors (subunits GluK4/5) deletion of the *iso*-propenyl group (CPAA) results in a 200-fold loss of binding affinity (1.6 μM). For KA, it has been suggested that a π–π interaction of the *iso*-propylene group with residue Tyr705 (GluK1 numbering) plays an important role for the high-affinity binding at the KA receptors.[33] As this structural feature is not present in CPAA and together with the fact that

Table 1. Chemical structures and binding affinities of selected Glu ligands at native AMPA, KA and NMDA receptors (rat brain synaptosomes) and at cloned homomeric rat GluK1–3.



Compd	Native receptors			Cloned homomeric receptors			
	AMPA IC_{50} [μM]	KA IC_{50} [μM]	NMDA K_i [μM]	GluA2 K_i [μM]	GluK1 K_i [μM]	GluK2 K_i [μM]	GluK3 K_i [μM]
SYM2081[a]	26.6	0.032	5.9	4.10 ± 0.27	0.000663 ± 0.000035	0.017 ± 0.003	0.0057 ± 0.0011
KA[b]	4	0.007	> 100[c]	3.69 ± 0.98	0.0759 ± 0.0122	0.0127 ± 0.0014	0.0328 ± 0.0049
DHK[c]	1100	6.0	350	> 1000	8.18 ± 0.29	12.3 ± 1.3	0.376 ± 0.060
CPAA[d]	106	1.6	0.5	–	–	–	–
DCAN[e]	> 300[i]	> 160[i]	> 300[i]	> 1000	> 100	> 100	> 100
BOAD	> 100[f]	14[f]	2.9[f]	> 100	0.667 ± 0.100	2.086 ± 0.072	0.625 ± 0.089
DOMO[g]	9.0	0.015	–	1.99 ± 0.23	0.0011 ± 0.0002	0.00604 ± 0.00104	0.00384 ± 0.00032

[a] References [21, 34]. [b] References [21, 22, 35]. [c] References [21, 36]. [d] Reference [37]. [e] Reference [27]. [f] Reference [38]. [g] References [21, 39]. Where – is shown, no data is available.

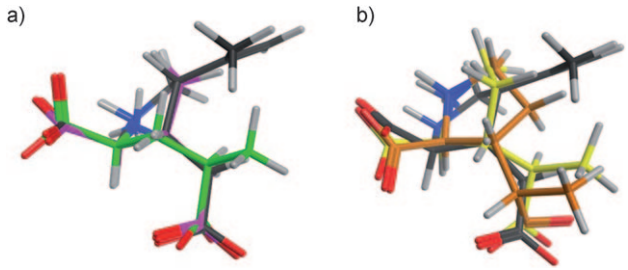
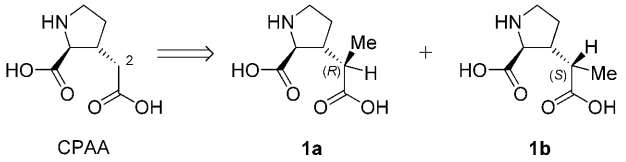


Figure 2. Three-point superimposition of calculated low-energy conformations (fitting atoms: ammonium and two carboxylate groups). a) KA (type code), CPAA (purple) and SYM2081 (green); b) KA (type code), **1a** (yellow) and **1b** (brown).

the binding affinity of SYM2081 at native KA receptors is as low as 32 nM (only fivefold lower than for KA), it is within reason to assume that such π–π interaction is not obligatory for high-affinity binding.

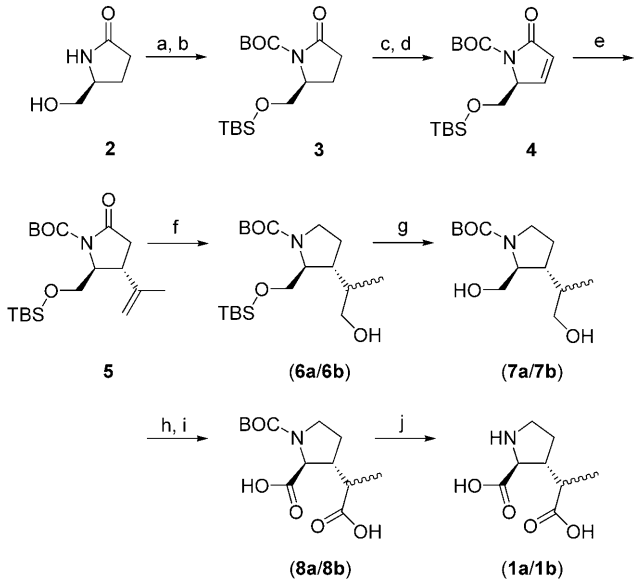
The question as to whether introduction of a methyl group in the 2-position of CPAA (corresponding to the 4-position of SYM2081) would restore the high binding affinity at the KA receptors is intriguing and also of general interest as the new analogues **1a**,**b** would represent a new class of KA receptor ligands (Scheme 1). Firstly, the low-energy conformation of the



Scheme 1. Chemical structures of CPAA and the rationally designed analogues (2*R*)-methyl-CPAA (**1a**) and (2*S*)-methyl-CPAA (**1b**).

two stereoisomers **1a**,**b** was determined by a stochastic conformational search. A subsequent three-point superimposition (Figure 2b) revealed that the (2*R*)-stereoisomer **1a** was optimal at representing the Glu folded conformations of KA and SYM2081, whereas the (2*S*)-stereosiomer **1b** posed a flipping of the envelope conformation of the pyrrolidine ring due to the disfavored interaction of the 2-methyl group with the C4 ring carbon.

A structural analysis of **1a** and **1b** suggests that the two target molecules may be looked at as highly functionalized proline derivatives[40] or conformationally restricted Glu analogues. Our retrosynthetic analysis advocates for (*S*)-pyroglutaminol (**2**) (Scheme 2) as the chiral starting synthon, which is readily obtained from (*S*)-pyroglutamate.[41] This strategy will secure the stereochemical configuration of the α-amino acid functionality and furthermore, as the key step, the 2,3-*trans*-configuration at C3 ring carbon may be fully controlled by an addition of a suitable cuprate to the protected enone **4**.[42] It seemed attractive to generate the cuprate from 2-bromopropene as the alkene would serve as a masked alcohol to be introduced in a one-pot procedure together with the planned reduction of the lactam by borane. The disadvantage of this



Scheme 2. Synthetic pathway towards the two stereoisomers of 2-Me-CPAA, **1a** and **1b**. *Reagents and conditions*: a) TBSCl, Et_3N, CH_2Cl_2; b) BOC_2O, Et_3N, DMAP, CH_2Cl_2 (87% two steps); c) LHMDS, THF, −78 °C, then PhSeCl; d) H_2O_2, EtOAc, 0 °C→RT (65% two steps); e) 2-bromopropene, *t*BuLi, CuCN, TMSCl, THF, −50 °C, then **4** (82%); f) BH_3·THF, reflux, then H_2O, NaOH, H_2O_2, 0 °C→RT (42%); g) TBAF, THF, RT (91%); h) Separation of the two stereoisomers **7a** and **7b** on HPLC (52%); i) $RuCl_3$, $NaIO_4$, H_2O, MeCN, EtOAc (59%); j) HCl/dioxane, RT (60%).

strategy is the lack of control of the stereochemistry at the vital C2-carbon. Thus, a successful separation of the two stereoisomers is required, preferably as the last step of the synthesis.

The projected synthesis of **1a**,**b** (Scheme 2) commenced with suitable protection of commercially available **2** to give **3** followed by introduction of the double bond to give **4**, according to literature procedures.[43] Copper-catalyzed conjugated addition of the *iso*-propenyl group to **4** gave the expected addition product **5** as a single enantiomer (2,3-*trans*) in high yield (82%).[38,42] The following one-pot borane-mediated reduction of the lactam carbonyl group and hydroboration of the alkene to give alcohols **6a/6b** proceeded in 42% yield with a 3:2 ratio of the two C2 stereoisomers. Removal of the *O*-TBS group was done smoothly with tetrabutylammonium fluoride (TBAF) under standard conditions to give diols **7a/7b**. Oxidation of the two alcohols to their corresponding carboxylic acids **8a/8b** was achieved by a catalytic amount of Ru^{III} with periodate as a co-oxidant, according to the modified Sharpless procedure.[44] Finally, deprotection of the amino group was done with hydrochloric acid in dioxane to give **1a/1b** in 60% yield.

Separation of the two C2 stereoisomers was monitored by HPLC for each step. While work efficiency would call for separation of stereoisomers as the final step, this was unfortunately not possible. It turned out that only the diastereomeric diols **7a/7b** displayed retention times of satisfactory separation. Thus, the synthesis was repeated with a separation step prior to oxidation of the diols to their corresponding carboxylic acids, as described in the Experimental Section and Scheme 2. The two diastereoisomers of the final products **1a/1b** were ini-

tially designated **S1** and **S2** awaiting C2 stereochemical assignment. To unquestionably determine the stereochemical configuration at C2, an X-ray structure study was planned for. However, despite extensive efforts varying the parameters (solvent and temperature), we were not successful and decided to proceed with the pharmacological characterization of **S1** and **S2**.

Pharmacological characterization of **S1** and **S2** commenced with binding affinity studies at native iGluRs (Table 2). For both stereoisomers, the affinity for AMPA receptors was without significance (>100 μM), whereas the binding affinity for KA receptors was found to be in the low micromolar range (2.4 and 1.8 μM, respectively). The main difference was observed for NMDA receptors as **S1** displayed a 22 μM binding affinity, whereas the binding affinity of **S2** was negligible (>100 μM). At the cloned homomeric KA receptors subtype GluK1–3 (Table 2), stereoisomer **S1** showed affinity for GluK1,3 in the low micromolar range (0.23 and 0.33 μM, respectively) with a 10- to 15-fold lower affinity for GluK2 (3.83 μM). This trend in binding affinity profile at GluK1–3 is often observed for KA ligands as exemplified by the Glu analogue BOAD (Table 1) and others.[20] However, for the **S2** stereoisomer the binding profile is different. For this analogue the binding affinity at GluK1–3 is within the same low micromolar range (0.39, 0.51 and 0.099 μM, respectively) with four- to fivefold in favor of GluK3.

Table 2. Pharmacological evaluation of stereoisomers **S1** and **S2** at native AMPA, KA and NMDA receptors and cloned homomeric subtypes GluK1-3.

Compd	Native receptors[a]			Cloned homomeric receptors[b]		
	AMPA	KA	NMDA	GluK1	GluK2	GluK3
		IC_{50} [μM]			K_i [μM]	
Stereoisomer S1	>100	2.4 [5.63 ± 0.06]	22 [4.65 ± 0.12]	0.23 ± 0.035	3.83 ± 0.54	0.33 ± 0.043
Stereoisomer S2	>100	1.8 [5.75 ± 0.02]	>100	0.39 ± 0.051	0.51 ± 0.020	0.099 ± 0.008

[a] All values were calculated from full concentration–response curves. For AMPA and KA: IC_{50} values with mean pIC_{50} ± SEM in brackets; For NMDA: K_i values with mean pK_i ± SEM in brackets. [b] K_i values reported are the mean ± SEM from at least three experiments, conducted in triplicate at 12–16 drug concentrations.

Conclusions

Two new conformationally restricted Glu analogues **1a/1b** were designed as hybrid structures of KA, CPAA and SYM2081. The two C2 stereoisomers were synthesized and subsequently underwent pharmacological evaluation as potential ligands at the iGluRs. In summary, the pharmacological profile of the **S1** stereoisomer at the iGluRs follows a trend often seen for iGluR ligands. That is a preference for native KA and NMDA over AMPA and a preference for cloned homomeric subtypes GluK1,3 over GluK2. On the other hand, the **S2** stereoisomer is fully selective for the KA receptors with negligible affinity for AMPA and NMDA receptors and similar affinities at the GluK1–3 subtypes. Given the pharmacological profile of the **S2** stereoisomer, this analogue of the two seems most promising as a lead structure for the discovery of new selective KA ligands by investigation of the chemical nature of the 2-substituent. However, the **S1** stereoisomer may hold potential for the further development into a selective NMDA ligand and an investigation of its subtype selectivity profile at this group of Glu receptors. Such plans are the topic of future work in our laboratories.

Experimental Section

Chemistry

All reagents were obtained from commercial suppliers and used without further purification. Solvents were dried by storing over 4 Å molecular sieves, except for THF which was distilled over sodium/benzophenone. Water- or air-sensitive reactions were conducted in flame-dried glassware under nitrogen using a syringe/septum cap technique. Purification by flash chromatography was done with silica gel size 40–63 μm (Merck, silica gel 60) or 35–70 μm (Fisher, silica 60 A). For TLC, Merck TLC silica gel F_{254} plates were used with appropriate spray reagents: $KMnO_4$ (0.5% in water), bromocresol green (0.1% in EtOH) or ninhydrin (0.06% in EtOH). ^{1}H NMR and ^{13}C NMR spectra were obtained on a Varian Mercury Plus (300 MHz) and a Varian Gemini 2000 instrument (75 MHz), respectively. HPLC was done using Agilent Prep HPLC systems with Agilent 1100 series pump, Agilent 1200 series diode array, multiple wavelength detector (G1365B), and Agilent PrepHT high performance preparative cartridge column (Zorbax, 300 SB-C18 Prep HT, 21.2×250 mm, 7 μm). MS spectra were recorded using LC-MS performed using an Agilent 1200 series solvent delivery system equipped with an autoinjector coupled to an Agilent 6400 series triple quadrupole mass spectrometer equipped with an electrospray ionization (ESI) source. Gradients of 10% aqueous acetonitrile + 0.05% formic acid (solvent A) and 90% aqueous acetonitrile + 0.046% formic acid (solvent B) were employed. Optical rotation was measured using a Perkin–Elmer 241 spectrometer, with a sodium lamp at 589 nm. Melting points were measured using an automated melting point apparatus, MPA100 OptiMelt (SRS) and are stated uncorrected.

(2*S*,3*R*)-*N*-*tert*-Butoxycarbonyl-2-(*tert*-butyldimethylsilyloxymethyl)-3-(*iso*-propenyl)-pyrrolidine-5 one (5): A solution of 2-bromopropene (0.67 mL, 7.60 mmol, 2.3 equiv) in dry THF (20 mL) at −78 °C was treated dropwise with *t*BuLi (1.7 M in pentane; 9.0 mL, 15.30 mmol, 4.7 equiv) and stirred for 15 min. Subsequently, a slurry of CuCN (342 mg, 3.80 mmol, 1.2 equiv) in dry THF (6 mL) was added via syringe, and the reaction mixture was allowed to reach −50 °C. After stirring at this temperature for 5 min, a clear, yellow solution was obtained and then recooled to −78 °C. Enone **4** (1.06 g, 3.25 mmol, 1.0 equiv) dissolved in dry THF (5 mL) was added dropwise followed by TMSCl (1.0 mL, 7.60 mmol, 2.3 equiv). The reaction mixture was allowed to reach −50 °C and stirred for 1 h. The solution was quenched with saturated aq NH_4Cl (50 mL) and extracted with EtOAc (3×100 mL). The combined organic phases were washed with brine (150 mL), dried ($MgSO_4$), filtered and evaporated to dryness. The crude product was purified by flash chromatography (EtOAc/heptane, 1:8; $R_f = 0.24$) to give **5** as a white solid (980 mg, 82%): mp: 35.1–35.9 °C; $[\alpha]_D^{23} = -50.60$; ^{1}H NMR ($CDCl_3$): $\delta = 4.78$ (ddd, 2H, $J = 5$, 3, 1 Hz), 3.93 (m, 2H), 3.73

(dd, 1H, $J=9$, 1 Hz), 2.93 (dd, 1H, $J=17.1$, 9.5 Hz), 2.84 (br d, 1H, $J=9$ Hz), 2.33 (dd, 1H, $J=17$, 1 Hz), 1.79 (s, 3H), 1.54 (s, 9H), 0.89 (s, 9H), 0.07 (s, 3H), 0.06 ppm (s, 3H); ^{13}CNMR ($CDCl_3$): $\delta=173.8$, 149.8, 145.7, 110.8, 82.8, 63.8, 63.5, 40.0, 37.6, 28.1, 25.8, 20.2, 18.2, −5.5, −5.5 ppm.

(*S*/*R*)-2-((2*S*,3*R*)-*N*-(*tert*-Butoxycarbonyl)-2-(*tert*-butyldimethylsilyloxymethyl)pyrrolidin-3-yl)propan-1-ol (6a/6b): A solution of **5** (1.55 g, 4.2 mmol, 1.0 equiv) in dry THF (50 mL) was treated at RT with BH_3·THF complex (1 M in THF; 25 mL, 25 mmol, 6.0 equiv), and the reaction mixture was refluxed (70 °C) for 4 h. After cooling to RT, an additional amount of THF (35 mL) was added and the reaction mixture was cooled to 0 °C. Water (3 mL), NaOH (2 M; 44 mL, 88 mmol, 21 equiv) and 30% *w*/*w* H_2O_2 in water (12 mL, 95 mmol, 23 equiv) were added carefully in this sequential fashion. The ice bath was removed and the reaction mixture was stirred for 1 h at RT, after which time it was quenched with saturated aq $NaHCO_3$ (100 mL). The aqueous phase was extracted with EtOAc (3× 150 mL) and the combined organic layers were washed with brine (200 mL), dried ($MgSO_4$), filtered and evaporated to dryness. The crude product was purified by flash chromatography (Et_2O/heptane, 2:1; $R_f=0.34$ and 0.25) to give a mixture of the two stereoisomers **6a** and **6b** (3:2) as a colorless oil (670 mg, 42%): ^{1}H NMR ($CDCl_3$), (two stereoisomers) $\delta=3.80$–3.40 (m, 6H, both isomers), 3.30–3.14 (m, 1H, both isomers), 2.43 (br s, 1H, minor isomer), 2.23 (m, 1H, major isomer), 2.07 (m, 1H, both isomers), 1.95 (m, 1H, major isomer), 1.78 (m, 1H, minor isomer), 1.66 (m, 1H, minor isomer), 1.47 (br s, 9H, both isomers), 0.98 (d, 3H, $J=7$ Hz, major isomer), 0.92 (d, 3H, minor isomer), 0.91 (br s, 18H, both isomers), 0.09 ppm (br s, 12H, both isomers); ^{13}C NMR ($CDCl_3$) (two diastereomers; rotamers in parentheses) $\delta=154.0$, 79.2, 78.8, 66.3, 65.9, 64.2, 63.4 (63.1), 61.6, 60.4 (60.3), 46.6, (46.5), 45.9 (45.8), 43.2 (42.4), 41.8 (41.4), 38.8 (38.6), 38.5 (38.3), 28.5, 28.3, 28.2, 27.0, 26.3, 25.9, 18.2, 14.3 (14.1), −5.4 ppm.

(*S*/*R*)-2-((2*S*,3*R*)-*N*-(*tert*-Butoxycarbonyl)-2-(hydroxymethyl)pyrrolidin-3-yl)propan-1-ol (7a/7b): A solution of **7a/7b** (750 mg, 2.0 mmol, 1.0 equiv) in dry THF (20 mL) was treated with TBAF (1 M in THF; 3.4 mL, 3.4 mmol, 1.7 equiv). The reaction mixture was stirred for 1 h at RT and then quenched with saturated aq $NaHCO_3$ (10 mL) and water (10 mL). The aqueous layer was extracted with EtOAc (3×50 mL), and the combined organic layers were washed with brine (150 mL), dried ($MgSO_4$), filtered, and evaporated to dryness. The crude product was purified by flash chromatography (EtOAc/heptane, 9:1; $R_f=0.25$), to give the two stereoisomers **7a** and **7b** (3:2) as a colorless oil (473 mg, 91%).

Separation of the two stereoisomers was carried out on HPLC with 15% CH_3CN in water as a mobile phase and a flow rate of 20 mL min^{-1} with UV detection at 200 nm. Stereoisomer **7a**: $t_R=$ 27.4 min; yield = 147 mg; *de* > 98%; $[\alpha]_D^{23}=-399.9$; ^{1}H NMR ($CDCl_3$): $\delta=3.65$–3.49 (m, 6H), 3.21 (m, 1H), 1.94 (m, 1H), 1.76 (m, 1H), 1.65 (m, 1H), 1.47 (s, 10H), 1.0 ppm (d, 3H, $J=7$ Hz); ^{13}C NMR ($CDCl_3$): $\delta=156.5$, 80.3, 67.1, 65.8, 62.0, 46.6, 43.0, 38.4, 28.6, 28.3, 15.0 ppm. Stereoisomer **7b**: $t_R=37.9$ min; yield = 124 mg; *de* = 98%; $[\alpha]_D^{23}=-307.5$; ^{1}H NMR ($CDCl_3$): $\delta=3.74$–3.46 (m, 6H), 3.15 (ddd, 1H, $J=10$, 7 Hz), 1.94 (m, 1H), 1.82 (m, 1H), 1.70 (m, 1H), 1.55 (m, 1H), 1.45 (s, 9H), 0.92 ppm (d, 3H, $J=7$ Hz); ^{13}C NMR ($CDCl_3$): $\delta=156.5$, 80.4, 66.8, 66.4, 63.1, 46.6, 42.9, 37.6, 28.6, 26.7, 13.8 ppm. Total yield after separation by HPLC: 52%.

(*S*/*R*)-2-((2*S*,3*R*)-*N*-(*tert*-Butoxycarbonyl)-2-(carboxy)pyrrolidin-3-yl)propanoic acid (8a,b): Stereoisomer **7a** (147 mg, 0.57 mmol, 1.0 equiv) was dissolved in EtOAc (4.3 mL) and CH_3CN (4.3 mL). $NaIO_4$ (995 mg, 4.65 mmol, 8.2 equiv) was dissolved in water (4 mL), and $RuCl_3$·H_2O (2.6 mg, 0.011 mmol, 0.02 equiv) was added. The aqueous phase was transferred to the organic mixture and water (2.2 mL) was added. The mixture was stirred at RT for 2.5 h. The mixture was filtered and the filter cake washed with EtOAc. The aqueous phase was extracted with EtOAc (10 mL) and the combined organic layers were washed with brine (15 mL), dried ($MgSO_4$), filtered and evaporated to dryness. The product was purified by flash chromatography (CH_2Cl_2/MeOH/AcOH, 100:5:2; $R_f=$ 0.25) to give **8a** as a white foam (96 mg, 59%): $[\alpha]_D^{23}=-516.6$; ^{1}H NMR ($CDCl_3$): $\delta=4.15$ (dd, 1H, $J=6$, 5 Hz), 3.60 (br m, 1H), 3.45 (br m, 1H), 2.70 (br m, 2H), 2.10 (m, 1H), 1.80 (m, 1H), 1.45 (d, 9H, $J=14$ Hz), 1.30 ppm (d, 3H, $J=7$ Hz); ^{13}C NMR ($CDCl_3$): (rotamers in parentheses) $\delta=180.2$ (180.0), 178.6, 155.3 (153.8), 81.2 (81.0), 61.6, 47.0 (46.0), 45.6 (45.5), 41.8 (41.6), 28.8 (28.3), 28.6 (28.5), 15.5 ppm (14.9).

Stereoisomer **7b** (100 mg, 0.35 mmol, 1.0 equiv) underwent the same oxidative reaction conditions as described above to give **8b** as a white foam (60 mg, 58%): $[\alpha]_D^{23}=-739.0$; ^{1}H NMR ($CDCl_3$): (rotamers in parentheses) $\delta=4.0$ (4.1) (dd, 1H, $J=8$, 5 Hz), 3.65 (2.28) (br m, 1H), 3.40 (3.55) (br m, 1H), 2.67 (b m, 1H), 2.53 (br m, 1H), 2.08 (2.0) (m, 1H), 1.64 (m, 1H), 1.45 (d, 9H, $J=13$ Hz), 1.25 ppm (d, 3H, $J=8$ Hz); ^{13}C NMR ($CDCl_3$): (rotamers in parentheses) $\delta=$ 180.2 (180.1), 178.0 (177.0), 155.1 (153.8), 81.2 (21.0), 63.6 (63.1), 46.8 (46.1), 45.8 (45.3), 42.4 (42.2), 28.6 (28.4), 28.2 (28.0), 15.4 ppm (15.1).

(*S*/*R*)-2-((2*S*,3*R*)-2-(Carboxy)pyrrolidin-3-yl)propanoic acid (1a/1b): For stereoisomer **S1**: **8a** (95 mg, 0.33 mmol, 1.0 equiv) was dissolved in dioxane (5 mL) and cooled to 0 °C. HCl (4 M in dioxane; 1 mL, 4.0 mmol, 12.1 equiv) was added dropwise, and the ice bath was removed and the solution stirred at RT for 18 h. The solvent was evaporated to dryness, and the compound purified by HPLC (mobile phase: 0.1% trifluoroacetic acid (TFA) in water, $t_R=$ 5.58 min.). The compound was dissolved in 1 M HCl and evaporated to dryness to give **S1** as the HCl-salt (36 mg, 60%): mp: 121.3–173.9 °C (decomposition); $[\alpha]_D^{23}=211.96$; ^{1}H NMR (D_2O): $\delta=4.29$ (dd, 1H, $J=7$, 3 Hz), 3.50–3.30 (br m, 2H), 2.70–2.60 (br m, 2H), 2.27 (m, 1H), 1.87 (m, 1H), 1.25 ppm (dd, 3H, $J=7$, 4 Hz); ^{13}C NMR (D_2O): $\delta=178.6$, 172.0, 61.7, 45.7, 45.0, 41.0, 27.7, 15.1 ppm; LC-MS (ESI): *m*/*z* $[M+H]^+$ calcd for $C_8H_{13}NO_4$: 188.08, found: 188.0.

For stereoisomer **S2**: **8b** (65 mg, 0.23 mmol, 1.0 equiv) was submitted to the same reaction conditions as described above for **S1**, to give **S2** as a white solid (25 mg, 60%, $t_R=6.69$ min): mp: 122.4–142.3 °C (decomposition); $[\alpha]_D^{23}=-0.806$; ^{1}H NMR (D_2O): $\delta=4.17$ (d, 1H, $J=6$ Hz), 3.50–3.30 (br m, 2H), 2.78 (m, 2H), 2.26 (1H), 1.87 (m, 1H), 1.22 (br d, 3H, $J=6$ Hz); ^{13}C NMR (D_2O): $\delta=179.2$, 171.5, 61.2, 45.7, 44.7, 41.3, 27.5, 14.0 ppm; LC-MS (ESI): *m*/*z* $[M+H]^+$ calcd for $C_8H_{13}NO_4$: 188.08, found: 188.0.

Modeling study

The modeling study was performed using the Molecular Operating Environment (MOE) software package (v2009.10; Chemical Computing Group, 2009) using the built-in mmff94x force field and the GB/SA continuum solvent model. The compound was submitted to a stochastic conformational search and with respect to its global minimum (ΔG in kcal mol^{-1}) returned conformations above +7 kcal mol^{-1} were discarded. 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. The superimposition of ligands was carried out using the built-in function in MOE, by fitting the ammonium group and the two carboxylate groups.

Pharmacology

Native receptor binding assays: Affinities for native AMPA, KA and NMDA receptors in rat cortical synaptosomes were determined using 5 nM (*RS*)-[^{3}H]AMPA (55.5 Ci mmol^{-1}),[45] 5 nM [^{3}H]KA (58.0 Ci mmol^{-1})[46] and 2 nM [^{3}H]CGP 39653 (K_d = 6 nM, 50.0 Ci mmol^{-1}),[47] respectively, with minor modifications as previously described.[48]

Recombinant receptor binding assays: Sf9 cells were cultured and infected with recombinant baculovirus of cloned rat GluA2(R)$_o$ or GluK1–3 and membranes prepared and used for binding as previously detailed.[21,49] The binding affinity of **1** was determined from competition experiments with 2–5 nM (*R*,*S*)-[^{3}H]AMPA (42.1 Ci mmol^{-1}; PerkinElmer, Waltham, MA, USA) at GluA2(R)$_o$ or 1–5 nM [^{3}H]SYM2081 (40 Ci mmol^{-1}; ARC, St. Louis, MO, USA) at GluK1(Q)$_{1b}$, GluK2(V,C,R)$_a$ and GluK3$_a$. Italic letters in parentheses indicate the RNA-edited isoforms of the subunits used. The IC_{50}, Hill coefficient and K_i values for **1** were evaluated as previously described.[50]

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Keywords: glutamate receptors · kainate · neurodegenerative diseases · neurotoxicity · neurotransmitters

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