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2-Amino-3-(3-hydroxy-1,2,5-thiadiazol-4-yl)propionic Acid: Resolution, Absolute Stereochemistry and Enantiopharmacology at Glutamate Receptors

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Abstract—In order to identify new subtype-selective (*S*)-glutamate (Glu) receptor ligands we have synthesized (*RS*)-2-amino-3-(3-hydroxy-1,2,5-thiadiazol-4-yl)propionic acid [(*RS*)-TDPA]. Resolution of (*RS*)-TDPA by chiral chromatography was performed using a Crownpac CR(+) column affording (*R*)- and (*S*)-TDPA of high enantiomeric purity (enantiomeric excess = 99.9%). An X-ray crystallographic analysis revealed that the early eluting enantiomer has *R*-configuration. Both enantiomers showed high affinity as well as high agonist activity at (*RS*)-2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid (AMPA) receptors, determined using a [^{3}H]AMPA binding assay and an electrophysiological model, respectively. The affinities and agonist activities obtained for (*R*)-TDPA ($IC_{50} = 0.265\,\mu M$ and $EC_{50} = 6.6\,\mu M$, respectively) and (*S*)-TDPA ($IC_{50} = 0.065\,\mu M$ and $EC_{50} = 20\,\mu M$, respectively) revealed a remarkably low AMPA receptor stereoselectivity, (*S*)-TDPA showing the highest affinity and (*R*)-TDPA the most potent agonist activity. In addition, (*S*)-TDPA was shown to interact with synaptosomal Glu uptake sites displacing [^{3}H](*R*)-aspartic acid ($IC_{50} \approx 390\,\mu M$). An enantiospecific and subtype-selective agonist activity was observed for (*S*)-TDPA at group I metabotropic Glu (mGlu) receptors ($EC_{50} = 13\,\mu M$ at $mGlu_5$ and $EC_{50} = 95\,\mu M$ at $mGlu_1$).

Introduction

Central excitatory amino acid pathways use (*S*)-glutamic acid (Glu) as neurotransmitter and appear to be implicated in a number of severe disease states for which no effective treatment currently exists. In addition to acute neurodegenerative disorders, these pathways probably are implicated in a number of chronic neurodegenerative conditions such as amyotrophic lateral sclerosis and Parkinson's and Alzheimer's diseases.[1–3] Glu activates ionotropic Glu (iGlu) receptors which mediate fast excitatory responses through cation channels as well as metabotropic Glu (mGlu) receptors mediating slower responses through G-proteins and second messenger systems.[3–5] Based on pharmacology, sequence similarities, and second messenger effector systems the mGlu receptors consist of three distinct, and pharmacologically interesting, groups: group I ($mGlu_1$/$mGlu_5$), group II ($mGlu_2$/$mGlu_3$), and group III ($mGlu_4$/$mGlu_6$/$mGlu_7$/$mGlu_8$), whereas the iGlu receptors are divided into three receptor subtypes named *N*-methyl-D-aspartic acid (NMDA), (*RS*)-2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid (AMPA) and kainic acid receptors.[3–5] Several subunits are known for each of the three iGlu receptor subtypes giving rise to a large number of native iGlu receptor isoforms, all of which may be of interest as therapeutic targets.

In order to characterize the individual receptor subtypes and to clarify their therapeutic significance subtype-selective agonists and antagonists are needed. AMPA (Fig. 1) is an example of a selective iGlu receptor agonist in which the distal carboxylic acid group of Glu has been replaced by an acidic heterocyclic moiety.[6] The sulfur analogue of AMPA, thio-AMPA, has been shown to have a pharmacological profile similar to that of AMPA, demonstrating that the 3-hydroxyisoxazole and the 3-hydroxyisothiazole units are effective carboxyl group bioisosteres.[7] With the aim of identifying new research tools with unique pharmacological properties we have focused on the 3-hydroxy-1,2,5-thiadiazole

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analogue of Glu (*RS*)-2-amino-3-(3-hydroxy-1,2,5-thiadiazol-4-yl)propionic acid [(*RS*)-TDPA] (Fig. 1). Though structurally similar, the three carboxyl group bioisosteres differ in terms of protolytic properties. The pK_a value of the distal carboxyl group of Glu is 4.3,[8] 3-hydroxy-1,2,5-thiadiazole and 3-hydroxyisoxazole have somewhat higher pK_a values (5.1 and 5.9, respectively),[9,10] and the pK_a value of 3-hydroxyisothiazole is markedly higher (7.5).[10] Previously, a preliminary report on (*RS*)-TDPA appeared as an abstract,[11] but neither the enantiomers nor the enantiopharmacology of (*RS*)-TDPA have been described. We here report a new synthesis of (*RS*)-TDPA, a chromatographic resolution of the compound, an absolute configurational determination of the enantiomers, and an enantiopharmacological characterization at iGlu as well as mGlu receptors.

Results

Chemistry

The synthesis of (*RS*)-TDPA is shown in Scheme 1. 3-Hydroxy-4-methyl-1,2,5-thiadiazole (**1**), prepared from (*RS*)-alanine amide hydrochloride and sulfur monochloride,[9] was protected using *tert*-butyl acetate under acid catalysis to give almost exclusively the *N*-alkylated compound (**2**). Subsequent NBS bromination of **2** gave rise to a separable mixture of the 4-mono- (**3**) and the 4-dibrominated compounds even when using only one equivalent of NBS. Alkylation of the sodium salt of dimethyl formamidomalonate with compound **3** afforded compound **4**, which was deprotected to give (*RS*)-TDPA.

Chromatographic resolution and X-ray crystallographic analysis

(*RS*)-TDPA was resolved into the (−)- and (+)-enantiomer using a semi-preparative Crownpak CR(+)-column[12] (Fig. 2) which previously has shown good enantioselectivity for a number of acidic amino acids containing primary amino groups.[12,13] After removal of the acidic mobile phase, the pure enantiomers could be crystallized as zwitterions with high enantiomeric excess (ee = 99.9%).

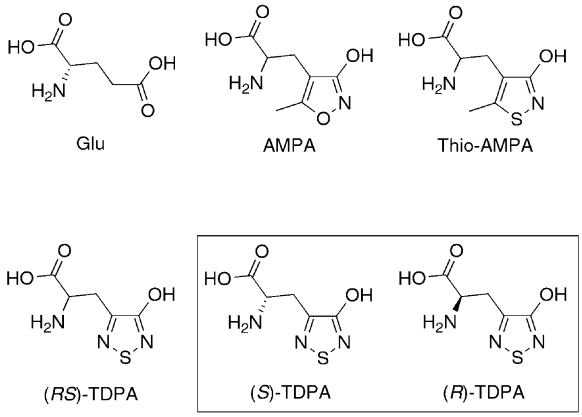
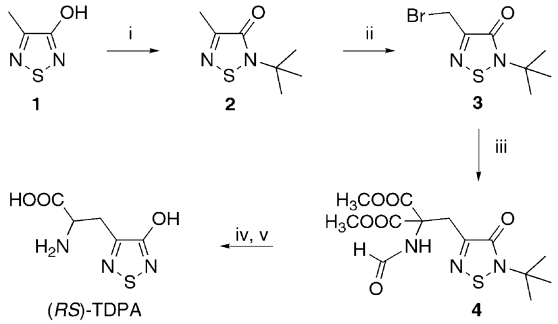


Figure 1. Structures of Glu, AMPA, thio-AMPA, (*RS*)-TDPA, and TDPA enantiomers.



Scheme 1. (i) *tert*-Butyl acetate, Amberlyst 15; (ii) NBS; (iii) $HCONHCH(COOCH_3)_2$, NaH; (iv) HBr (4 M), TFA; (v) Et_3N.

Based on the elution order of a number of both natural and unnatural α-amino acids on the Crownpak CR(+) column, the first eluting (−)-enantiomer was expected to have the *R*-configuration.[12] An X-ray crystallographic analysis of this enantiomer confirmed the *R*-configuration (Fig. 3). The Flack absolute structure parameter was calculated to $x = -0.001(12)$.[15,16]

Pharmacology at iGlu receptors and synaptosomal Glu uptake

The affinities for AMPA, kainic acid, and NMDA receptors were determined using the radioligands [^{3}H]AMPA,[17] [^{3}H]kainic acid,[18] or [^{3}H](*R*)-3-(2-carboxy-4-piperazinyl)propyl-1-phosphonic acid[19] ([^{3}H]CPP) (Table 1). Neither racemic nor enantiopure TDPA showed affinity for NMDA or kainic acid receptors. (*S*)-TDPA was found to possess approximately twice the affinity of (*RS*)-TDPA at AMPA receptors, whereas (*R*)-TDPA was two fold weaker than the racemate (Fig. 4 and Table 1). In contrast, (*S*)-TDPA was 3-fold weaker than (*R*)-TDPA in the rat cortical wedge preparation ($EC_{50} = 20\,\mu M$ and $EC_{50} = 6.6\,\mu M$, respectively), thus showing the opposite order of potency

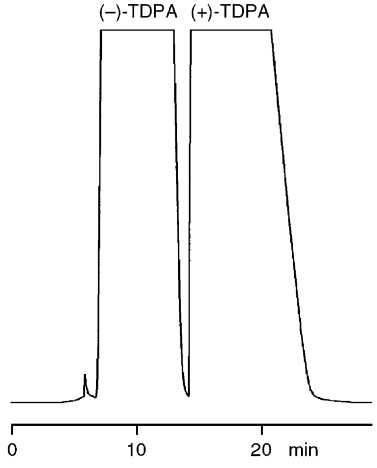


Figure 2. Chromatographic resolution of (*RS*)-TDPA (7 mg) on Crownpak CR(+) (150×10 mm) using aqueous trifluoroacetic acid (pH 2.0) as mobile phase at 1 °C.

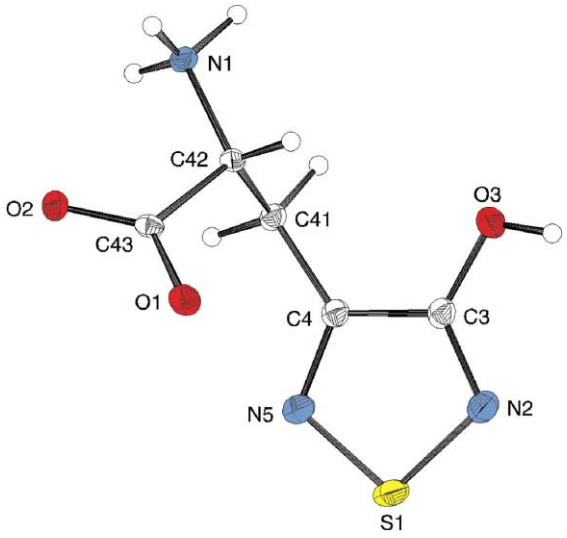


Figure 3. The molecular structure (ORTEP II[14]) of (*R*)-(−)-TDPA. Displacement ellipsoids are shown at the 50% probability level. Hydrogen atoms are represented by spheres of arbitrary size.

compared to the binding experiment. The effects of (*RS*)-, (*R*)-, and (*S*)-TDPA on synaptosomal Glu uptake using [^{3}H](*R*)-aspartic acid as a substrate were studied using a previously described method.[20] Whereas no inhibitory effects could be detected for (*RS*)-TDPA or (*R*)-TDPA (both at 200 μM), (*S*)-TDPA (200 μM) reduced [^{3}H](*R*)-aspartic acid uptake by 33±2% (corresponding to IC_{50} = 390±30 μM).

Pharmacology at mGlu receptors

(*RS*)-TDPA displayed agonist activity at both $mGlu_{1\alpha}$ (EC_{50} = 210 μM) and $mGlu_{5a}$ (EC_{50} = 36 μM), whereas it was inactive when tested as an agonist or an antagonist at $mGlu_2$ and $mGlu_{4a}$ representing group II and III, respectively (Table 1). (*S*)-TDPA was twice as potent as (*RS*)-TDPA at $mGlu_{1\alpha}$ (EC_{50} = 95 μM) and $mGlu_{5a}$ (EC_{50} = 13 μM) being 7- and 3-fold less potent than Glu, respectively (Table 1 and Fig. 5). Like the racemate, (*S*)-TDPA was inactive at $mGlu_2$ and $mGlu_{4a}$ (Table 1). (*R*)-TDPA was inactive at all four mGlu receptor subtypes both when tested as agonist and as antagonist, showing a pharmacological profile similar to that of AMPA (Table 1 and Fig. 5).

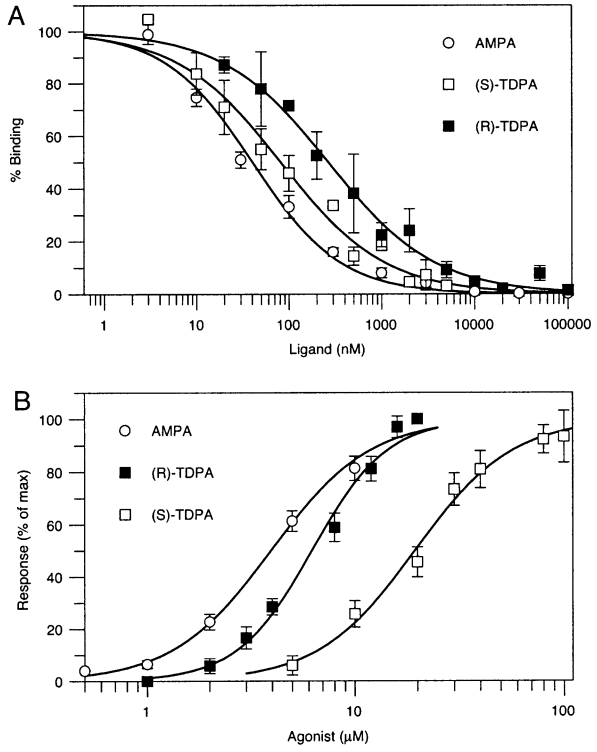


Figure 4. Displacement of [^{3}H]AMPA receptor binding (A) and concentration–response curves from the rat cortical wedge preparation (B) for AMPA and (*S*)- and (*R*)-TDPA. Data points are the mean (±SD) of at least three individual experiments.

Discussion

In order to obtain subtype-selective receptor ligands and useful pharmacological research tools, a number of analogues of Glu have been reported in recent years, in which the distal carboxyl group of Glu has been replaced by hydroxylated 5-membered heteroaromatic moieties.[3,7,21] We here describe the preparation and stereochemistry of the two enantiomers of the 3-hydroxy-1,2,5-thiadiazole analogue of Glu, (*RS*)-TDPA, and an enantiopharmacological characterization at iGlu and mGlu receptors.

(*RS*)-TDPA was shown to possess a pharmacological profile at iGlu receptors similar to that of the structurally related compounds, AMPA and thio-AMPA, all compounds being selective AMPA receptor agonists.

Table 1. Rat membrane receptor binding, rat cortical wedge electrophysiology, and pharmacology at cloned mGlu receptors expressed in CHO cells[a]

	IC_{50} (μM)			EC_{50} (μM)	EC_{50} (μM)			
	[^{3}H]AMPA	[^{3}H]Kainic acid	[^{3}H]CPP	Electrophysiology	$mGlu_{1\alpha}$	$mGlu_{5a}$	$mGlu_2$	$mGlu_{4a}$
Glu	0.34±0.10	0.38±0.11	0.11±0.00	>1000	13±2	4.4±0.8	4.8±1.0	13±1
AMPA[b]	0.040±0.014	>100	>100	3.5±0.2	>1000	>1000	>1000	>1000
TDPA	0.12±0.04	>100	>100	13±1	210±32	36±8	>1000	>1000
(*S*)-TDPA	0.065±0.015	>100	>100	20±0.9	95±10	13±2	>1000	>1000
(*R*)-TDPA	0.27±0.05	>100	>100	6.6±0.7	>1000	>1000	>1000	>1000

[a]Values are the mean±SEM of at least three independent experiments.
[b]iGlu receptor data from ref 39.

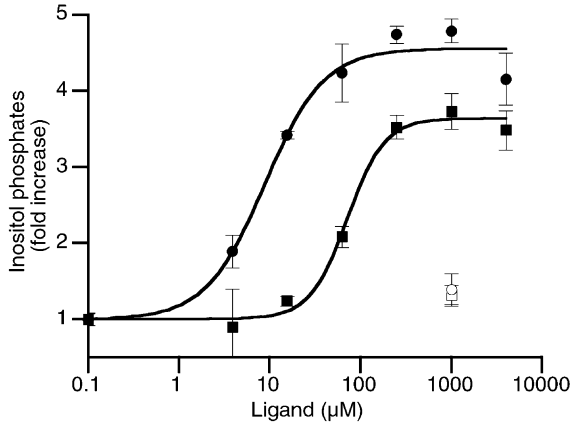


Figure 5. Concentration–response curves for the agonist (*S*)-TDPA at CHO-cells expressing $mGlu_{1\alpha}$ (■) and $mGlu_{5a}$ (●) and AMPA at CHO-cells expressing $mGlu_{1\alpha}$ (□) and $mGlu_{5a}$ (○). Data-points are the mean (±SD) of representative experiments performed in triplicate.

These observations are in agreement with an earlier preliminary report.[11] However, in contrast to AMPA and other AMPA receptor agonists,[22] TDPA quite surprisingly was shown to interact with AMPA receptors with a low degree of stereoselectivity. Based on [^{3}H]AMPA receptor binding studies, the eudismic ratio for TDPA is approximately 4, (*S*)-TDPA being the eutomer (Table 1 and Fig. 4), which is far less than what is generally observed for known enantiopure AMPA receptor ligands.[22,23] Based on electrophysiological data, obtained from the rat cortical wedge preparation, (*R*)-TDPA (ee = 99.9%) appears to be the eutomer. This difference in eudismic ratio based on affinity and activity data is most likely a reflection of the presence of transport systems in the cortical wedge preparation. Thus, (*S*)-TDPA, but not (*R*)-TDPA, interacts with the synaptosomal Glu uptake system as shown in the present study and with Glu transporter protein, EAAT2, reported in an earlier study.[24] This enantioselective interaction with Glu uptake is likely to explain that (*S*)-TDPA shows lower potency than (*R*)-TDPA in the cortical wedge preparation.

Compared to the inactivity as AMPA receptor agonists of the *R*-enantiomers of known enantiopure Glu analogues containing 3-hydroxyisoxazole or 3-hydroxyisothiazole moieties,[25–28] the agonist activity of (*R*)-TDPA is noteworthy. Thus, AMPA receptors exhibit an unprecedentedly low stereoselectivity towards the enantiomers of (*RS*)-TDPA. We suggest that (*R*)-TDPA, in contrast to the known 3-hydroxyisoxazole- or 3-hydroxyisothiazole-containing Glu analogues with *R*-configuration, in the absence of a substituent in the 5-position of the heterocyclic ring can adopt a receptor-active agonist conformation with no serious energy penalty.

The enantiopharmacological profile of TDPA at mGlu receptors was less complex than that observed for the iGlu receptors, (*S*)-TDPA being a selective group I mGlu receptor agonist at $mGlu_1$ and $mGlu_5$, 7 and 3 times less active than Glu, respectively. The activation of these mGlu receptor subtypes elicited by (*S*)-TDPA was highly stereoselective, since (*R*)-TDPA was inactive as an agonist as well as an antagonist at all mGlu receptors tested. This demonstrates a unique mGlu receptor selectivity for the 3-hydroxy-1,2,5-thiadiazole analogue of Glu.

Conclusion

In comparison with 5-substituted 3-hydroxyisoxazoles and 3-hydroxyisothiazoles, the 3-hydroxy-1,2,5-thiadiazole has been shown to be a unique carboxyl group bioisostere for the distal carboxyl group of Glu at iGlu as well as mGlu receptors. TDPA and its enantiomers possess a distinct mGlu receptor selectivity as well as a unique stereoselectivity at iGlu receptors. Based on the atypical AMPA receptor affinity and activity, (*R*)- and (*S*)-TDPA are interesting new leads and tools for Glu receptor research.

Experimental

Chemistry

General procedures. Dimethylformamide (DMF) was dried using 3 Å molecular sieves. Column chromatography (CC) was performed on silica gel 60, 0.063–0.200 mm (Merck). Melting points were determined in capillary tubes and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AC-200F spectrometer at 200 and 50 MHz, respectively. Unless otherwise stated, $CDCl_3$ was used as solvent. Chemical shifts are given in parts per million (ppm) with respect to the residual solvent peak unless otherwise stated. IR spectra were recorded from KBr discs on a Perkin-Elmer 781 grating infrared spectrophotometer. Elemental analyses were performed by Analytical Research Department, H. Lundbeck A/S, Denmark. Optical rotations were measured in thermostated cuvettes on a Perkin-Elmer 241 polarimeter. CD spectra were recorded in 0.1 M HCl solution in 1.0 cm cuvettes at room temperature on a Jasco J-720 spectropolarimeter.

Liquid chromatography. Preparative chiral HPLC was performed using a Crownpak CR(+) column (150×10 mm) equipped with a Crownpak CR(+) guard column (10×4.0 mm) (Daicel) connected to a Jasco 880 pump, a Rheodyne 7125 injector with a 5 mL loop, a Waters M481 spectrophotometer (254 nm), and a Hitachi D-2000 Chromato-Integrator. The column was eluted at 0 °C (ice bath) with aqueous TFA (pH 2.0) at 1.5 mL/min. Enantiomeric excess (ee) was determined using a Crownpak CR(−) column (150×4.0 mm) (Daicel), connected to a Waters M510 pump, a Waters U6K injector, and a Waters 991 photodiode array detector. The column was thermostated at 1 °C with a Hetofrig thermostat and eluted with aqueous $HClO_4$ (pH 2.0) at 0.4 mL/min. The ee was determined from peak areas. Ion-exchange chromatography was performed using S-Sepharose Fast Flow (Pharmacia) packed in a Pharmacia HR-10/10 column (ion capacity: 1.4 mmol), washing the column first with 2M aqueous AcOH and then eluting with H_2O at 1.0 mL/min. The column was

connected to the HPLC system mentioned above for the preparative chiral HPLC.

2 - *tert* - Butyl - 4 - methyl - 1,2,5 - thiadiazol - 3 - one (2). 3-Hydroxy-4-methyl-1,2,5-thiadiazole[9] (**1**) (2.00 g, 17 mmol) was dissolved in *tert*-butyl acetate (50 mL, 0.37 mol) and Amberlyst 15 (0.1 g) was added. The reaction mixture was then placed inside a stainless steel pressure reactor which was heated to 80 °C for 14 h. The bomb was cooled to −10 °C and then opened. The suspension was filtered and evaporated. The residue was dissolved in Et_2O (50 mL) and washed with aqueous NaOH (50 mL, 2 M) and with H_2O (50 mL) and then dried ($MgSO_4$). Upon filtration and evaporation, CC (CH_2Cl_2 to CH_2Cl_2/MeOH, 19:1) afforded compound **2** (2.12 g, 71%) as a low melting solid. An analytical sample was distilled (108–110 °C, 15 mm Hg): ^{1}H NMR (TMS): δ 2.24 (s, 3H), 1.58 (s, 9H). ^{13}C NMR: δ 162.3, 155.9, 59.2, 27.4, 16.4. Anal. Calcd for $C_7H_{12}N_2OS$: C, 48.81%; H, 7.02%; N, 16.26%. Found: C, 48.93%; H, 7.06%; N, 16.06%.

4-Bromomethyl-2-*tert*-butyl-1,2,5-thiadiazol-3-one (3). Compound **2** (2.00 g, 11 mmol), NBS (2.17 g, 12.2 mmol), and dibenzoyl peroxide (0.14 g, 0.5 mmol) was refluxed in CCl_4 (60 mL) for 16 h. After cooling, light petroleum (100 mL) was added and the reaction mixture was filtered. The filtrate was evaporated and subjected to CC (CH_2Cl_2) which gave compound **3** (2.04 g, 73%) as an oil which solidified upon standing: ^{1}H NMR (TMS): δ 4.36 (s, 2H), 1.65 (s, 9H). ^{13}C NMR: δ 160.2, 152.4, 59.7, 27.2, 23.0. Compound **3** was used without further purification.

Methyl 3-(2-*tert*-butyl-3-oxo-1,2,5-thiadiazol-4-yl)-2-formamido-2-methoxycarbonyl propionate (4). To a solution of dimethyl 2-formamidomalonate[29] (1.19 g, 6.8 mmol) in dry DMF (50 mL) a dispersion of NaH in oil (0.27 g, 60%, 6.8 mmol) was added. Upon stirring of this suspension for 30 min under N_2, a solution of **3** (1.63 g, 6.5 mmol) in dry DMF (100 mL) was added and the stirring was continued for 16 h at rt. After evaporation of the mixture, EtOAc (200 mL) was added. The organic phase was washed with H_2O (2×200 mL), dried ($MgSO_4$), filtered, and finally evaporated to dryness. Recrystallization of the residue (toluene/heptane) afforded compound **4** (1.42 g, 63%): ^{1}H NMR (DMSO-d_6): δ 8.85 (s, 1H), 7.91 (s, 1H), 3.69 (s, 6H) 3.45 (s, 2H), 1.53 (s, 9H). ^{13}C NMR (DMSO-d_6): δ 167.2, 161.5, 161.1, 152.9, 63.8, 59.0, 53.6, 32.8, 26.9. Anal. calcd for $C_{13}H_{19}N_3O_6S$: C, 45.21%; H, 5.55%; N, 12.17%; S, 9.28%. Found: C, 45.56%; H, 5.59%; N, 12.40%; S, 9.28%.

(*RS*)-2-Amino-3-(3-hydroxy-1,2,5-thiadiazol-4-yl)propionic acid [(*RS*)-TDPA]. In a sealed flask, compound **4** (485 mg, 1.40 mmol) was dispersed in aqueous HBr (22 mL, 4 M). TFA (1 mL) was added and the suspension was heated at 70–80 °C for 18 h. The solution obtained was evaporated to dryness, the residue was re-evaporated three times from H_2O and dried in vacuo over KOH and P_2O_5. The residue was added H_2O and a small amount of insoluble material was removed by filtration. Upon treatment with activated charcoal and evaporation, the residue was dissolved in EtOH (99.9%) and the solution adjusted to pH 3 using Et_3N (200 μL). The precipitate was recrystallized (H_2O) to give (*RS*)-TDPA (153 mg, 58%): mp >250 °C. IR: 3300–2500 (m, several bands), 1645 (s), 1530 (s), 1515 (s), 1430 (s), 1400 (s), 1330 (s), 1315 (s), 1285–1275 (s) cm^{-1}. ^{1}H NMR (D_2O, acetonitrile): δ 4.11 (m, 1H), 3.25 (m, 2H). Anal. Calcd for $C_5H_7N_3O_3S$: C, 31.74%; H, 3.73%; N, 22.21%. Found: C, 31.58%; H, 3.72%; N, 22.19%. The mother liquor furnished after two recrystallizations an additional 16.5 mg of (*RS*)-TDPA (total yield: 64%).

(*R*)-(−)-2-Amino-3-(3-hydroxy-1,2,5-thiadiazol-4-yl)propionic acid [(*R*)-TDPA] and (*S*)-(+)-2-amino-3-(3-hydroxy-1,2,5-thiadiazol-4-yl)propionic acid [(*S*)-TDPA]. A solution of (*RS*)-TDPA (256 mg, 1.35 mmol) in aqueous TFA (64 mL, pH 2.0) was filtered through a 0.45 μm filter (Millex-HV, Millipore) to remove any particles and resolved on a Crownpak CR(+) column in 40 injections. All the fractions of the first eluting enantiomer were pooled, evaporated, and reevaporated three times from H_2O. In order to remove traces of TFA, the oily residue was redissolved in 2 M aqueous AcOH and subjected to ion-exchange chromatography. Appropriate fractions were pooled, evaporated, and the crystalline residue was reevaporated three times from H_2O. Recrystallization (H_2O) gave (*R*)-TDPA (73 mg, 57%) as white crystals: mp >250 °C; 99.9% ee; $[\alpha]_D^{29}$ −16.3 (*c* 0.34, 0.1 M HCl); Δε (210 nm) −0.2 m^2/mol. IR: 3300–2400 (m, several bands), 1620 (s), 1545 (s), 1515 (s), 1430 (s), 1405 (s), 1335 (s), 1290 (s) cm^{-1}. ^{1}H NMR (D_2O, acetonitrile): δ 4.11 (m,1H), 3.26 (m, 2H). All fractions containing the second enantiomer were pooled, evaporated, re-evaporated twice from H_2O, and dried in vacuo. The crystalline residue was recrystallized from H_2O to give (*S*)-TDPA (108 mg, 85%): mp >250 °C; 99.9% ee; $[\alpha]_D^{27}$ +16.3 (*c* 0.36, 0.1 M HCl); Δε (210 nm) +0.2 m^2/mol. The ^{1}H NMR and IR spectra of (*S*)-TDPA were identical with those of the *R*-enantiomer. Anal. Calcd for $C_5H_7N_3O_3S$: C, 31.74%; H, 3.73%; N, 22.21%. Found: C, 31.74%; H, 3.65%; N, 22.15%.

X-ray crystallography

(*R*)-(−)-2-Amino-3-(3-hydroxy-1,2,5-thiadiazol-4-yl)propionic acid [(*R*)-TDPA]. Single crystals were grown by slowly cooling a heated aqueous solution of (*R*)-TDPA. Crystal data: $C_5H_7N_3O_3S$, $M_r = 189.20$, monoclinic, space group $P2_1$ (No. 4), $a = 7.4098(8)$ Å, $b = 5.843(2)$ Å, $c = 8.8964(9)$ Å, $\beta = 96.640(9)°$, $V = 382.6(1)$ Å^3, $Z = 2$, $D_c = 1.642$ Mg m^{-3}, $F(000) = 196$, μ(Cu K_α) = 3.584 mm^{-1}, $T = 122.0$ (5) K, crystal dimensions = 0.45×0.32×0.10 mm.

Data collection and processing. Diffraction data were collected on an Enraf-Nonius CAD-4 diffractometer using graphite monochromated Cu K_α radiation (λ = 1.54184 Å).[30] Intensities were collected using the ω/2θ scan mode. Unit cell dimensions were determined by least squares refinement of 20 reflections (θ range 41.78–44.22°).[30] The reflections were measured in the range

$-9 \le h \le 9$, $-7 \le k \le 7$, $-11 \le l \le 11$, ($5.00° < \theta < 74.62°$). Data were reduced using the programs of Blessing (DREADD).[31,32] The intensities of five standard reflections were monitored every 10^4 s (decay 3.6%, corrected). Absorption correction was applied using the programme ABSORB ($T_{min} = 0.375$; $T_{max} = 0.715$).[33] A total of 3170 reflections were averaged according to the point group symmetry 2 resulting in 1566 unique reflections ($R_{int} = 0.0238$ on F_o^2).

Structure solution and refinement. The structure was solved by the direct method using the programme SHELXS97[34,35] and refined using the programme SHELXL97.[16] Full matrix least-squares refinement on F^2 was performed, minimizing $\Sigma w\left(F_o^2 - F_c^2\right)^2$, with anisotropic displacement parameters for the non-hydrogen atoms. The positions of the hydrogen atoms were located on intermediate difference electron density maps and refined with fixed isotropic displacement parameters. Correction for extinction was applied [coefficient: 0.077(2)]. The refinement (132 parameters, 1566 reflections) with the molecule having the *R*-configuration converged at $R_F = 0.0183$, $wR_{F^2} = 0.0507$ for 1566 reflections with $F_o > 4\sigma(F_o)$; $w = 1/[\sigma^2(F_o^2) + (0.0242P)^2 + 0.1065\ P]$, where $P = (F_o^2 + 2F_c^2)/3$; $S = 1.111$. In the final difference Fourier map maximum and minimum electron densities were 0.236 and -0.182 e Å^{-3}, respectively. Refinement of the Flack absolute structure factor x in the final refinement gave $x = -0.001(12)$.[15,16] Complex atomic scattering factors for neutral atoms were as incorporated in SHELXL97.[16,36] Crystallographic data (excluding structure factors) for (*R*)-(−)-TDPA has been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC 174364. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44–1223–336033 or e-mail: deposit@ccdc.cam.ac.uk).

In vitro pharmacology

Receptor binding. The membrane preparation used in all receptor binding experiments were prepared according to the method described by Ransom and Stec.[37] Affinities for NMDA, AMPA and kainic acid receptors were determined using [^{3}H]CPP[19] (5 nM), [^{3}H]AMPA[17] (5 nM), and [^{3}H]kainic acid[18] (5 nM) with the modifications described below. All binding experiments were carried out at 0–4 °C. On the day of experiments, frozen homogenates were quickly thawed and re-suspended in 50 volumes of buffer (pH 7.4) (50 mM Tris–HCl + 2.5 mM $CaCl_2$, 30 mM Tris–HCl + 2.5 mM $CaCl_2$, or 50 mM Tris–HCl, for [^{3}H]CPP, [^{3}H]AMPA, or [^{3}H]kainic acid binding, respectively), and centrifuged (48,000*g*, 10 min). This step was repeated four times. In [^{3}H]AMPA binding experiments, 100 mM KSCN was added to the buffer during the final wash and during incubation. The final pellet was re-suspended in ice-cold buffer, corresponding to approximately 50, 25, or 25 mg/mL original tissue for [^{3}H]CPP, [^{3}H]AMPA, or [^{3}H]kainic acid binding, respectively. Non-specific binding was determined using 1 mM Glu. For [^{3}H]CPP binding, aliquots consisted of 50 μL test solution, 50 μL [^{3}H]CPP solution, and 400 μL membrane suspension. Following incubation for 30 min, filtration (GF/B) was carried out through a 48-well Brandell Cell Harvester, followed by washing with 3×0.5 mL buffer. Filters were added 3 mL of Opti-Flour, and the amount of bound radioactivity was determined using a TRI-CARB liquid scintillation analyzer. [^{3}H]AMPA and [^{3}H]kainic acid binding were carried out in aliqouts consisting of 25 μL [^{3}H]ligand solution, 25 μL test solution, and 200 μL membrane suspension. Binding was terminated by filtration through GF/B filters using a 96-well Packard FilterMate Cell Harvester and washing with 3×250 μL buffer. Filters were dried and added 25 μL Microscint 0. The amount of bound radioactivity was determined using a Packard TOPCOUNT microplate scintillation counter. The data was analyzed using Grafit 3.0, Leatherbarrow software. Data were fitted to the equation: $B = 100 - (100 \times [\text{Inhibitor}]^n)/(IC_{50}^n + [\text{Inhibitor}]^n)$, where B is the binding as a percentage of total specific binding, and n the Hill coefficient.

Synaptosomal Glu uptake. The experiments were carried out using rat cortical synaptosomes prepared from male Sprague–Dawley rats (200–249 g) essentially as previously described.[20] The final synaptosomal pellet was re-suspended in assay buffer containing 128 mM NaCl, 10 mM glucose, 5 mM KCl, 1.5 mM NaH_2PO_4, 1.77 mM $CaCl_2$, 1 mM $MgSO_4$, and 10 mM Tris (pH 7.4) in a final concentration of 2 mg/mL. The experiment was carried out at ambient temperature. The assay was initiated by adding 800 μL membrane solution to a mixture of test substance (100 μL) and a solution (100 μL) containing [^{3}H](*R*)-aspartic acid (30 nM) and (*R*)-aspartic acid (3 μM). Following a 10 min incubation, the reaction was terminated by filtering through 48-well Brandell cell harvester using GF/B filters. Filters were washed with 3×3 mL 50 mM Tris buffer. Total binding was determined in the presence of buffer, and non-specific binding was determined in the presence of 1 mM (*R*)-aspartic acid.

In vitro electrophysiology. The rat cortical wedge preparation[38] in a modified version[39] was used for determination of the depolarizing effects of the compounds under study. Agonists were applied for 90 s. Receptor selectivity was determined by antagonizing responses, approximately corresponding to the EC_{50} values of the compounds in question, with 5 μM CPP and 5 μM 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(F)-quinoxaline (NBQX) for NMDA and AMPA receptors, respectively. Antagonists were applied for 90 s, followed by co-application of agonist and antagonist for 90 s. The data were fitted to the equation: % response = $(E_{max} \times [\text{Agonist}]^n)/(EC_{50}^n + [\text{Agonist}]^n)$, where E_{max} is the relative maximal response and n is the Hill coefficient.

Cell culture. The Chinese hamster ovary (CHO) cell lines stably expressing $mGlu_{1\alpha}$, $mGlu_2$, $mGlu_{4a}$ and $mGlu_{5a}$ receptors have previously been described.[40–43] They were maintained at 37 °C in a humidified 5% CO_2 incubator in Dulbecco's Modified Eagle Medium (DMEM) containing a reduced concentration of (*S*)-

glutamine (2 mM) and were supplemented with 1% (*S*)-proline, penicillin (100 U/mL), streptomycin (100 mg/mL) and 10% dialyzed fetal calf serum (all GIBCO, Paisley, Scotland, UK). Two days before assay, 1.8×10^6 or 1×10^6 cells were divided into the wells of 48-well plates ($mGlu_{1\alpha}$ and $mGlu_{5a}$) or 96-well plates ($mGlu_2$ and $mGlu_{4a}$), respectively.

Second messenger assays. PI hydrolysis was measured as described previously.[44,45] Briefly, the cells were labeled with [^{3}H]inositol (2 μCi/mL) 24 h prior to the assay. For agonist assays, the cells were incubated in phosphate buffered saline (PBS)-LiCl for 20 min followed by a 20 min incubation in PBS-LiCl containing ligand, and agonist activity was determined by measurement of the level of [^{3}H]-labeled mono-, bis- and tris-inositol phosphates by ion-exchange chromatography. For antagonist assays, the cells were pre-incubated with the ligand dissolved in PBS-LiCl for 20 min prior to incubation with ligand and 30 μM Glu for 20 min. The antagonist activity was then determined as the inhibitory effect of the Glu mediated response. The assay of cyclic AMP formation was performed as described previously.[44,45] Briefly, the cells were pre-incubated in PBS containing 1 mM 3-isobutyl-1-methylxanthine (IBMX) and then incubated for 10 min in PBS containing the ligand, 10 μM forskolin, and 1 mM IBMX (both Sigma Chemicals, St. Louis, MO, USA). The agonist activity was then determined as the inhibitory effect of the forskolin-induced cyclic AMP formation. For antagonist assay, the cells were pre-incubated with ligand dissolved in PBS containing 1 mM IBMX for 20 min prior to a 10 min incubation in PBS containing the ligand, 30 μM Glu, 10 μM forskolin, and 1 mM IBMX. Cyclic AMP levels were determined by use of a SPA kit (Amersham).

Supporting Information Available

Tables for (*R*)-(−)-2-amino-3-(3-hydroxy-1,2,5-thiadiazol-4-yl)propionic acid [(*R*)-TDPA] listing crystal data and structure refinement, final atomic coordinates and equivalent isotropic displacement parameters, anisotropic displacement parameters for non-hydrogen atoms, and a full list of bond lengths, bond angles, torsion angles and hydrogen bond dimensions (7 pages); lists of structure factors (4 pages).

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