Experimental Section

Melting points were determined using an Electrothermal 8103 apparatus and are uncorrected. IR spectra were taken with Perkin-Elmer 398 and FT 1600 spectrophotometers. ^{1}H-NMR spectra were recorded on a Bruker 200 MHz spectrometer with TMS as internal standard; the values of the chemical shifts (d) are given in ppm and coupling constants (*J*) in Hz. All reactions were carried out under argon atmosphere. Progress of the reaction was monitored by TLC on silica gel plates (Riedel-de-Haen, Art. 37341). Merck silica gel (Kieselgel 60) was used for chromatography (70-230 mesh) and flash chromatography (230-400 mesh) columns. Extracts were dried over $MgSO_4$, and solvents were removed under reduced pressure. Elemental analyses were performed on a Perkin-Elmer 240C elemental analyzer, and the results are within ±0.4% of the theoretical values. Yields refer to purified products and are not optimized.

Cbz-Glu(α-OMe)-Ser(OMe) (20a).

A solution of N-Cbz-L-Glutamic acid-α-methylester **18a** (2.5 g, 8.5 mmol) in CH_2Cl_2 (60 ml) was cooled to 0°C , and dicyclohexylcarbodiimide (1.9 g, 9.3 mmol) and hydroxybenzotriazole (1.25 g, 9.3 mmol) were added. The reaction mixture was maintained to 0 °C for 1h, and then it was allowed to warm to room temperature. L-serine methylester hydrocloride **19a** (2 g, 9.3 mmol) and triethylamine (1.5 ml, 10.2 mmol) were added to the reaction mixture that was stirred for 12 h.

The reaction mixture was filtered to remove DCU. The organic layer was washed with 20 ml of saturated $NaHCO_3$ solution and 20 ml of 10% aqueous citric acid solution and then was washed with brine, dried, and concentrated *in vacuo*. The residue was purified by flash chromatography (EtOAc) to give 3 g (89 %) of **20a** as white solid (mp 103-105°C).

$[\alpha]_D$=+ 43° (c = 1 , $CHCl_3$), ^{1}H-NMR ($CDCl_3$) δ 7.31 (s, 5 H), 6.69 (br s, 1 H), 5.77 (m, 1 H), 5.06 (s, 2 H), 4.64-4.60 (m, 1 H), 4.39-4.31 (m, 1 H), 3.89-3.87 (m, 2 H), 3.74 (s, 3 H), 3.71 (s, 3 H), 2.37-2.25 (m, 2 H), 1.90-1.67 (m, 2 H). Anal Calcd for $C_{18}H_{24}N_2O_8$: C,H,N.

(2S,4'S)-2-(Benzyloxycarbonylamino)-4-(4'-methoxycarbonyloxazolin-2-yl)butyric Acid Methyl Ester (21a).

A solution of **20a** (3 g, 7.5 mmol) in anhydrous THF (70 ml) was degassed and (2.16 g, 9.09 mmol) of Burgess reagent was added. The reaction mixture was refluxed for 1h and then quenched with 20 ml of water. The aqueous layer was extracted with EtOAc (3 x 30 ml) and the combined organic layers were washed with brine, dried, and concentrated in vacuo. The residue was purified by flash chromatography (8:2, EtOAc and hexanes) to give 1.2 g (42%) of **21a** as a colorless oil. $[\alpha]_D$ = + 62.6° (c = 3.91, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 7.32 (s 5 H), 5.63 (NH), 5.08 (s 2H), 4.72-4.63 (m 1H), 4.46-4.30 (m 2H), 3.72 (s 6H), 2.42-2.26 (m 2H), 2.22-1.99 (m 2H). Anal Calcd for $C_{18}H_{22}N_2O_7$: C,H,N.

(2S)-2-Amino-4-(4'-hydroxycarbonyloxazol-2-yl)butyric Acid (2).

A solution of **21a** (500 mg, 1.32 mmol) in CH_2Cl_2 (6 ml) was cooled to 0 °C , and $BrCCl_3$ (136 µl, 1.38 mmol) and DBU (295 µl, 1.98 mmol) were added. The reaction mixture was maintained at 0°C overnight and then quenched with 5 ml of water. The aqueous layer was extracted with dichloromethane (3 x 5 ml) and the combined organic layers were washed with brine, dried and concentrated in vacuo. The residue was purified by flash chromatography (8:2 EtOAc and hexanes) to give 213 mg (43%) of oxidized intermediate.

$[\alpha]_D$=+15.3° (c = 4.26 $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ: 8.06 (s, 1 H), 7.27 (s, 5 H), 5.63 (br s, 1 H), 5.03 (s, 2 H), 4.46-4.36 (m, 1 H), 3.82 (s, 3 H), 3.86 (s, 3 H) , 2.88-2.80 (m, 2 H), 2.45-2.01 (m, 2 H).

To a solution of oxidized product (213 mg , 0.56 mmol) in THF-H_2O 1:1 (10 ml), LiOH 40 (mg, 1.68 mmol) was added. The reaction mixture was stirred at room temperature overnight. The THF was evaporated in vacuo and the aqueous layer was washed with EtOAc (1 x 10 ml) prior to the addition of a 1N HCl (pH=3). The aqueous layer was extracted with EtOAc (3 x 5 ml) and the combined organic layers were washed with brine, dried and concentrated in vacuo.

The resulting product was dissolved in CH_3OH (10 ml) and 10% Pd-C (20 mg) was added. The suspension was stirred for 1h under a hydrogen atmosphere (1 atm).Then the mixture was filtered through a plug of Celite and the filtrate was washed with water 4 times. Water was removed by liophilization to give **2** (110 mg, 90%) as a white solid. $[\alpha]_D$=+8.3° (c = 0.72, H_2O); ^{1}H-NMR (D_2O) δ 8.22 (s 1 H), 3.80-3.73 (m 1 H), 2.93-2.85 (m 2 H), 2.28-2.24 (m 2 H). Anal Calcd for $C_8H_{10}N_2O_5$: C,H,N.

Cbz-Asp(α-OBn)-Ser(OBn) (20b).

A solution of N-Cbz-L-Aspartic acid-α-benzylester **18b** (1 g, 2.8 mmol) in CH_2Cl_2 (40 ml) was cooled to 0 °C, and dicyclohexylcarbodiimide (0.64 g, 3.08 mmol) and hydroxybenzotriazole (0.4 g, 3.08 mmol) were added. The reaction mixture was maintained to 0 °C for 1h, and then it was allowed to warm to room temperature. L-serine benzyl ester hydrochloride **19b** (0.7 g, 3.08 mmol) and triethylamine (468 µl, 3.36 mmol) were added to the reaction mixture that was stirred for 12 h at rt. The reaction mixture was filtered to remove DCU. The organic layer was washed with 20 ml of saturated $NaHCO_3$ solution and 20 ml of 10% aqueous citric acid solution and then was washed with brine, dried, and concentrated in vacuo. The residue was purified by flash chromatography (2:1 EtOAc and hexanes) to give 1.2 g (80 %) of **20b** as white solid: mp 118-120°C; $[\alpha]_D$=+24° (c = 0.63 $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ: 7.31 (s, 15 H), 6.52 (br s, 1 H), 6.04 (m, 1 H), 5.18 (s, 2 H), 5.17 (s, 2 H), 5.09 (s,

2 H), 4.68-4.59 (m, 2 H), 3.95-3.70 (m,, 2 H), 3.01 (dd, 1 H, J = 4.4, 16.3 Hz), 2.77 (dd, 1 H, J = 4.8, 16.2 Hz). Anal Calcd for $C_{29}H_{30}N_2O_8$: C,H,N.

(2S,4'S)-2-(Benzyloxycarbonylamino)-3-(4'-benzyloxycarbonyloxazolin-2-yl)propionic Acid Benzyl Ester (21b).

A solution of **20b** (1.2 g, 2.25 mmol) in anhydrous THF (60 ml) was degassed and (0.64 g, 2.7 mmol) of Burgess reagent was added. The reaction mixture was refluxed for 1 h and then quenched with 30 ml of water. The aqueous layer was extracted with EtOAc (3 x 30 ml) and the combined organic layers were washed with brine, dried and concentrated in vacuo. The residue was purified by flash cromatography (1:1, EtOAc and hexanes) to give 465 mg (40%) of **21b** as a colorless oil.

$[\alpha]_D$=+ 33° (c = 1.13, $CHCl_3$); ^{1}H-NMR($CDCl_3$) δ 7.31 (s 15 H), 6.06(NH), 5.15(s 2 H), 5.11(s 2 H), 5.10(s 2 H), 4.77-4.73 (m 1 H), 4.58-4.49 (m 1 H), 4.35-4.21 (m 2 H), 3.00;(dd 1 H J=4.4 J= 16.2), 2.78 (dd 1 H J= 4.5 J= 16.15); MS (m/z): 65, 91(100), 319, 337, 381, 516 (M^+). Anal Calcd for $C_{29}H_{28}N_2O_7$: C,H,N.

(2S,4'S)-2-Amino-3-(4'-hydroxycarbonyloxazolin-2-yl)propionic Acid (1a).

A solution of **21b** (0.4 g, 0.75 mmol) in CH_3OH (20 ml), 10% Pd-C (40 mg) was added. The suspension was stirred for 3h under a hydrogen atmosphere (1 atm). Then the mixture was filtered through a plug of Celite and the solvent was concentrated in vacuo to give **1a** in 93% yield as a white solid. $[\alpha]_D$=+10.9 (c = 1.1, H_2O); ^{1}H-NMR (D_2O) δ: 4.45-4.36 (m 1 H), 3.93-3.91 (m 2 H), 2.92-2.89 (m 2H); ^{13}C-NMR(D_2O) δ: 175.670, 173.749, 173.635, 66.389, 56.319, 53.361, 37.437; MS (FAB) m/z 203 (MH^+). Anal Calcd for $C_7H_{10}N_2O_5$: C,H,N.

Cbz-Glu(α-OBn)-Ser(OBn) (20c).

A solution of N-Cbz-L-Glutamic acid-α-benzyl ester (2.5 g, 6.73 mmol) in CH_2Cl_2 (70 ml) was cooled to 0°C , and dicyclohexylcarbodiimide (1.5 g, 7.4 mmol) and hydroxybenzotriazole (1 g, 7.4 mmol) were added. The reaction mixture was maintained to 0 °C for 1h, and then it was allowed to warm to room temperature. L-serine benzyl ester hydrochloride **19b** (1.6 g, 6.73 mmol) and triethylamine (1.1 ml , 8.0 mmol) were added to the reaction mixture that was stirred for 12 h.

The reaction mixture was filtered to remove DCU. The organic layer was washed with 35 ml of saturated $NaHCO_3$ solution and 35 ml of 10% aqueous citric acid solution and then dried and concentrated in vacuo. The residue was purified by flash chromatography (6:4 EtOAc and hexanes) to give 3.5 g (95 %) of **20c** as white solid: mp 88-90 °C; $[\alpha]_D$=+22° (c = 0.68, $CHCl_3$); H-NMR ($CDCl_3$) δ 7.31 (s, 15 H), 6.60 (br s, 1 H), 5.76 (m, 1 H), 5.17 (s, 2 H); 5.14 (s, 2 H), 5.04 (s, 2 H), 4.67-4.63 (m, 1 H), 4.44-4.40 (m, 1 H), 3.94 (br s, 2 H), 2.5-1.98 (m, 4 H). Anal Calcd for $C_{30}H_{32}N_2O_8$: C,H,N.

(2S,4'S)-2-(Benzyloxycarbonylamino)-4-(4'-benzyloxycarbonyloxazolin-2-yl)butyric Acid Benzyl Ester (21c).

A solution of **20c** (3.5 g, 6.3 mmol) in anhydrous THF (80 ml) was degassed and 1.8 g (7.5 mmol) of Burgess reagent was added. The reaction mixture was refluxed for 1h and then quenched with 30 ml of water. The aqueous layer was extracted with EtOAc (3 x 40 ml) and the combined organic layers were washed with brine, dried and concentrated in vacuo. The residue was purified by flash chromatography (1:1 EtOAc and hexanes) to give 1.5 g (45%) of **21c** as a colourless oil.

$[\alpha]_D$=+ 40° (c = 1.14, $CHCl_3$); ^{1}H-NMR($CDCl_3$) δ 7.33 (s, 15 H), 5.70 (br s, 1 H), 5.22 (s, 4 H), 5.08 (s, 2 H), 4.73-4.64 (m, 1 H), 4.48-4.31 (m, 3 H), 2.41-2.08 (m, 4 H). Anal Calcd for $C_{30}H_{30}N_2O_7$: C,H,N.

(2S,4'S)-2-Amino-4-(4'-hydroxycarbonyloxazolin-2-yl)butyric Acid (1b).

A solution of **21c** (0.45 g, 0.84 mmol) in CH_3OH (20 ml), 10% Pd-C (40 mg) was added. The suspension was s tirred for 3 h under a hydrogen atmosphere (1 atm). Then the mixture was filtered through a plug of Celite and the solvent was concentrated in vacuo to give **1b** in 95% yield as a white solid. $[\alpha]_D$=+ 7.95° (c = 2.51, H_2O); ^{1}H-NMR (D_2O) δ 4.46-4.38 (m, 1 H), 4.20-4.17 (m, 1 H), 3.84-3.82 (m, 2 H), 2.89-2.81 (m, 2 H), 2.5-2.38 (m, 2 H), 2.14-2.01 (m, 2 H); ^{13}C-NMR (D_2O) δ 178.6, 174.9, 172.3, 64.8, 63.9, 62.6, 32.8, 27.7; MS (FAB) m/z 217 (MH^+). Anal Calcd for $C_8H_{12}N_2O_5$: C,H,N.

(4S,5S)-2-Phenyloxazoline-4,5-dicarboxylic Acid Dibenzyl Ester (23a).

To a stirred solution of (2S)-N-benzoyl-L-aspartic acid dibenzyl ester **22a** (208 mg, 0,5 mmol) in dry THF (20 ml) was added LHMDS (1 ml, 1 M solution in THF) in one portion, under argon at –78 °C. After 2 h, at the same temperature, a solution of iodine (254 mg, 1 mmol) in dry THF (10 ml) was added dropwise. The reaction was quenched after 1 h with 10 ml of an aqueous saturated solution of NH_4Cl and the solvent was removed under reduced pressure. The residue was dissolved in ethyl acetate and washed with a solution of $Na_2S_2O_3$. The organic layer was washed with brine, dried and concentrated. Flash cromatography on silica gel of the residue (hexane/ethyl acetate 9:1 as eluent) gave oxazoline **23a** (166 mg) in 70% yield. ^{1}H NMR ($CDCl_3$) δ 5.02 (d, 1 H, J = 6.5 Hz), 5.24 (s, 2 H), 5.27 (s, 2 H), 5.41 (d, 1 H, J = 6.5 Hz), 7.33 (s, 10 H), 7.44-7.52 (m, 3 H), 7.90 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 52.9, 53.1, 72.8, 78.0, 126.1, 128.4, 128.7, 131.9, 167.3, 170.0. Anal Calcd for $C_{25}H_{21}NO_5$: C,H,N.

(4S,5S)-2-Phenyloxazoline-4,5-dicarboxylic Acid (3).

The oxazoline **23a** (166 mg, 0.4 mmol) was dissolved in CH_3OH (10 ml) and 10% Pd-C (20 mg) was added. Then the suspension was stirred for 1h under a hydrogen atmosphere (1 atm). Then the mixture

was filtered through a plug of Celite and the filtrate was washed with methanol 5 times. The solvent was removed under reduced pressure to give **3** (85 mg, 90%) as a white solid essentially pure. ^{1}H NMR (DMSO-d_6) δ 4.78 (d, 1 H, J= 6.2 Hz), 5.24 (d, 1 H, J = 6.2 Hz), 7.45-7.52 (m, 3 H), 7.85 (m, 2 H); ^{13}C NMR (DMSO-d_6) δ 55.6, 70.0, 129.3, 129.4, 131.8, 133.9, 164.7, 167.7, 172.7; $[\alpha]_D$ = +6.8° (c 2.9, DMSO). Anal Calcd for $C_{11}H_9NO_5$: C,H,N.

(4S,5S)-2-Phenyloxazoline-4,5-dicarboxylic Acid Dimethyl Ester (23b).

Following a similar procedure as described for **23a**, starting from (2S)-N-benzoyl-L-aspartic acid dimethyl ester **22b** (265 mg, 1 mmol), the product **23b** was obtained. Flash chromatography (8:2 hexanes and EtOAc) gave the pure oxazoline **23b** (210 mg) in 80% yield as a white solid. ^{1}H NMR ($CDCl_3$) δ 3.83 (s, 3 H), 3.85 (s, 3 H), 5.03 (d, 1 H, J = 6.6 Hz), 5.45 (d, 1 H, J = 6.6 Hz), 7.30-7.50 (m, 3 H), 7.90 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 52.9, 53.1, 72.8, 78.0, 126.1, 128.4, 128.7, 131.9, 167.3, 170.0; $[\alpha]_D$ = +42.2° (c 1.2, $CHCl_3$). Anal Calcd for $C_{13}H_{13}NO_5$: C,H,N.

2-Phenyloxazole-4,5-dicarboxylic Acid Dimethyl Ester (24).

The oxazoline **23b** (300 mg, 1.14 mmol) was dissolved in benzene (20 ml); when the solution refluxed, NiO_2 (154 mg, 1.7 mmol) was added and the suspension was stirred overnight at the same temperature. The reaction mixture was then cooled, ethyl acetate (40 ml) was added, and this solution was washed with water. The organic layer was washed with brine, dried and concentrated in vacuo. Flash chromatography of the residue (8:2 hexanes and ethyl acetate) gave the product **24** (208 mg) in 70% yield as colourless prisms. ^{1}H NMR ($CDCl_3$) δ 3.94 (s, 3 H), 3.99 (s, 3 H), 7.82-7.90 (m, 3 H), 8.13 (m, 2 H). Anal Calcd for $C_{13}H_{11}NO_5$: C,H,N.

2-Phenyl-oxazole-4,5-dicarboxylic Acid (4).

To a solution of the oxazole **24** (0.56 mmol) in THF-H_2O 1:1 (10 ml), LiOH (40 mg, 1.68 mmol) was added. The reaction mixture was stirred at room temperature overnight. The THF was evaporated under reduced pressure and the aqueous layer was washed with EtOAc (1 x 10 ml) prior to the addition of a 1N solution of HCl until pH=3. The aqueous layer was extracted with EtOAc (3 x 5 ml) and the combined organic layers were washed with brine, dried and concentrated under reduced pressure to give **4** (120 mg, 93%) as colourless prisms. ^{1}H NMR (DMSO-d_6) δ 7.50-7.62 (m, 3 H), 8.00 (m, 2 H). Anal Calcd for $C_{11}H_7NO_5$: C,H,N.

Computational Chemistry.

All molecular modelling was runned on a Silicon Graphics Indigo2 workstation. Systematic conformational searches were carried out on the structures reported in Chart 1B,C[1] as well as on newly desiged Glu/Asp derivatives (Chart 1A) using the SEARCH routine within Sybyl 6.6 (Tripos). All torsional angles were scanned with 30° increments within a 0°-359° Range. In the case of torsional angles about amidic bonds, the incremenent was set to 180°. Whenever the torsional angles were associated to the rotation of a phenyl ring, the absolute interval of variation was restricted to 180°. Starting conformations were considered with zeroed torsional angles. A 0.750 Van der Waals Radii Scaling Factors was used to soften steric contacts in the rigid rotamers. Torsional angles included in rings were analysed using the Ring Search module, by increment of 10° using 0°-359° as interval of variation. the permissible variance on the distance between the ring closure atoms was set to 0.3 Å, while the permissible variance on the valence angles about the ring closure atoms was set to 10°. In the case of known ligands distance maps were created, starting from the most rigid compounds of each set: Cis-1-amminocyclobutane-1,3-dicarboxylate (cis-ACBD) (transportable substrates) and L-anti-endo-3,4-methanopyrrolidine-3,4-dicarboxylate (L-anti-endo-3,4-MPDC) (non transportable inhibitors).

Carboxylic carbons and the protonable nitrogen were considered as distance map points with a grid size of 0.5 Å. Pharmacophore-consistent conformations were searched using distance maps as constrain on the basis of the Marshall's active analog approach[2]. All theoretically possible conformations were generated without evaluating their conformational energy. Resulting structures files were transferred in Insight98 (MSI) software to perform energetic calculations. All the conformations were geometrically optimized (Discover module) using the cvff force field[3], considering the aminoacidic functions charged. Energy minimizations were performed in vacuo ($\varepsilon = 1*r$) with a distance dependent dielectric constant using conjugate gradient as minimization algorithm (Fletcher, R. Unconstrained Optimization In *Pratical Methods of Optimization* John Wiley & Sons: New York 1980, Vol.1.) until the maximum RMS derivative was less than 0.001 kcal/mol. Resulting output conformers were clustered into a smaller number of families according to the values of their torsional angles. Conformers, whose energy differs more than 5 kcal/mol from the global minimum, were rejected. Molecular superimpositions and volume manipulations were performed respectively with the FIT and MVOLUME commands within Sybyl 6.6.

Table 1. Distances Between the two Carboxylic Carbons of Substrates and Non Transported EAAT Inhibitors and RMSD Values for their Pharmacophoric Points Superimposition on ACBD and 2,3-MPDC, Respectively.

Substrates	Distance (Å)	RMSD (Å)	Non transported inhibitors	Distance (Å)	RMSD (Å)
2,4-PDC	4.8	0.094	**2,3-PDC**	3.8	0.39
2,4-MPDC	4.9	0.09	**2,3-MPDC**	4.7	/
L-t-3-OH-Asp	3.9	0.34	**L-t-3-BnOAsp**	3.7	0.33
CCGIII	4.0	0.34	**L-t-3-BzAsp**	3.9	0.4
ACBD	4.7	/	**Dihydrokainate**	4.2	0.45
4-OH-Glu	4.3	0.18	**Kainate**	3.7	0.69
1a	4.6	0.9	**3**	3.5	0.53
1b	6.1	1.3	**4**	3.4	0.59
2	6.4	0.9			

Binding Assay[4].

Membrane Preparation

Male rats (CRL:CD(SD) BR-COBS, Charles River) were killed by decapitation, cortices were rapidly dissected from other brain regions and used immediatly for membrane preparation.

[^{3}H]AMPA, [^{3}H]Kainic acid and [^{3}H]CGP 39653 binding

The tissue was homogenized using an Ultra Turrax TP-1810 in the following ice cold buffers Tris HCl, 30 mM, pH 7.1 containing 2.5 mM $CaCl_2$, for [^{3}H]AMPA binding; Tris acetate, 50 mM, pH 7.1 containing 2.5 mM $CaCl_2$ for [^{3}H]Kainic acid binding; Tris HCl 5 mM, pH 8 for [^{3}H]CGP 39653 binding.

The homogenate was centrifuged at 48000xg for 10 min at 4°C (three times for [^{3}H]AMPA and [^{3}H]Kainic binding, once for [^{3}H]CGP 39653 binding). After centrifugations the pellet was resuspended in the same fresh buffer (with 10 mM EDTA for [^{3}H]CGP 39653 binding) and incubated at 37°C for 30 min (10 min for [^{3}H]CGP 39653 binding). The suspension was then centrifuged at 48000xg for 10 min at 4°C, the supernatant discarded and the pellet was homogenized in the same fresh buffer (without EDTA for [^{3}H]CGP 39653 binding), than frozen at -20°C. For [^{3}H]CGP 39653 binding one additional centrifugation was done, and the remaing pellet was then frozen at -20°C.

[^{3}H]MDL 105,519 binding

Rat cerebral cortex was homogenized in a Potter-Elvejeim homogenizer with a teflon pestle in 10 vols of ice-cold 0.32 M sucrose, pH 7.4 and centrifuged at 1000xg for 10 min. The supernatant was collected and centrifuged at 20000xg for 20 min and the resulting pellet was resuspended in 20 vols of fresh water, kept 15 min in ice and then centrifuged at 8000xg for 20 min The supernatant and the "buffy-coat" were carefully collected and centrifuged at 48000xg for 20 min The pellet obtained was stored at -20°C until the day of the binding assay.

Binding Assay

[^{3}H]AMPA binding.

On the day of assay, the frozen tissue suspension was thawed and centrifuged at 48000xg for 10 min at 4°C. The pellet was resuspended in fresh Tris HCl, 30 mM, pH 7.1 containing 2.5 mM $CaCl_2$

and centrifuged again, the pellet obtained was finally resuspended in 50 vols of Tris HCl, 30 mM, pH 7.1 containig 2.5 mM $CaCl_2$ and 100 mM KSCN.

[^{3}H]AMPA binding was done in a final incubation volume of 0.5 ml consisting of 0.25 ml of membrane suspension (10 mg of tissue/sample), 0.25 ml [^{3}H]AMPA (s.a. 40 Ci/mmol, NEN; final concentration 5 nM) and 10 µl of displacing agents or solvent; non specific binding was obtained in presence of 10 µM AMPA. Incubation (30 min at 4°C) was stopped by rapid filtration under vacuum (Brandell MR 48R) through GF/C filters which were then washed with 12 ml of ice-cold buffer. The filters were placed in vials containing 4 ml of liquid scintillation (Ultima Gold MV, Packard) and counted in a LKB 1214 rackbeta scintillation counter.

[^{3}H]Kainic acid binding

On the day of assay, the frozen tissue suspension was thawed and centrifuged at 48000xg for 10 min at 4°C. The pellet was resuspended in 6.5 vols of 5 mM EGTA, incubated for 10 min at 4°C and centrifuged again as above; than the pellet obtained was resuspended in Tris acetate, 50 mM, pH 7.1 and washed two times. The pellet obtained is finally resuspended in 120 vols of Tris acetate, 50 mM, pH 7.1.

[^{3}H]Kainic acid binding was done in a final incubation volume of 1ml consisting of 0.5 ml of membrane suspension (8 mg of tissue/sample), 0.5 ml [^{3}H]Kainic acid (s.a. 58 Ci/mmol, NEN; final concentration 2 nM) and 20 µl of displacing agents or solvent; non specific binding was obtained in presence of 1 µM Kainic acid. Incubation (60 min at 4°C) was stopped by rapid filtration under vacuum (Brandell MR 48R) through GF/C filters which were then washed with 12 ml of ice-cold buffer and counted as above.

[^{3}H]CGP 39653 binding to NMDA receptor

On the day of assay, the frozen pellet was thawed and washed two times by resuspension in Tris HCl, 5 mM, pH 7.7 and centrifugation at 48000xg for 10 min at 4°C. The pellet obtained is finally resuspended in 100 vols of the same fresh buffer.

[^{3}H]CGP 39653 binding was done in a final incubation volume of 1 ml consisting of 0.5 ml of membrane suspension (10 mg of tissue/sample), 0.5 ml [^{3}H]CGP 39653 (s.a. 48.9 Ci/mmol, NEN; final concentration 2 nM) and 20 µl of displacing agents or solvent; non specific binding was obtained in presence of 100 µM L-glutamic acid. Incubation (60 min at 4°C) was stopped by rapid filtration under

vacuum (Brandell MR 48R) through GF/B filters which were then washed with 12 ml of ice-cold buffer and counted as above.

[^{3}H]MDL 105,519 binding to Glycine site

On the day of assay, the frozen pellet was thawed and washed 4 times by resuspension in Tris acetate, 50 mM, pH 7.4 and centrifugation at 48000xg for 10 min at 4°C. The pellet obtained was finally resuspended in 100 vols of the same fresh buffer.

[^{3}H]MDL 105,519 binding was done in a final incubation volume of 0.5 ml consisting of 0.25 ml of membrane suspension (5 mg of tissue/sample), 0.25 ml [^{3}H]MDL 105,519 (s.a. 69 Ci/mmol, Amersham; final concentration 4 nM) and 10 µl of displacing agents or solvent; non specific binding was obtained in presence of 1 mM glycine. Incubation (30 min at room temperature) was stopped by rapid filtration under vacuum (Brandell MR 48R) through GF/B filters which were then washed with 12 ml of ice-cold buffer and counted as above.

Dose-inhibition curves were analyzed by the "Allfit" program to obtain the concentration of unlabeled drug that caused 50% inhibition of ligand binding (IC_{50}); the data were processed with the Cheng and Prusoff equation to obtain the Ki values.

Cytotoxicity Assay[5]. Materials.

Mice (CD1 strain) were obtained from Charles River UK Ltd (Kent, UK). Cell culture plasticware was obtained from Nunc A/S (Roskilde, Denmark) and Corning (Corning, NY). Foetal calf serum was supplied by Sera-Lab Ltd. (Sussex, UK). All other chemicals were of the purest grade available from regular commercial sources [Merck Ltd. (Dorset, UK); Sigma Chemical Co. (St. Louis, MO); Fluka Chemicals Ltd. (Dorset, UK)].

Cerebellar Granule Cell Cultures.

Primary cultures of cerebellar granule cells were prepared as described previously (Schousboe et al., 1989). Using this method, it has been established that this culture comprises at least 95% granule cells. Briefly, cerebella from 7-day-old CD1 mice were dissociated by mild trypsinization and subsequent trituration in a solution containing soybean trypsin inhibitor and DNAse. Dissociated cells were pelleted (5 min at 1000 rpm) and resuspended in Dulbecco's modified Eagle medium supplemented

with 19.5 mM KCl, 24 mM glucose, 2 mM glutamine, 7 μM *p*-amino-benzoic acid, insulin (100 mU/L), penicillin G (5 x 10^5 U/L) and foetal calf serum (10% v/v; heat inactivated). Cells were seeded in 96-well plates (25 x 10^6 cells per plate) pre-coated with a 1% (w/v) solution of poly-L-lysine and cultured at 37^0C in a humidified atmosphere of 5% CO_2/95% air. Experiments were performed on cells after 7 days in culture (7-DIV). Cytosine arabinoside (20 μM final concentration) was added 48-hr (for 7-DIV cultures) after plating to prevent glial proliferation.

Assessment of Cytotoxicity.

Cytotoxicity was assessed by a spectrophotometric method (Balazs et al., 1988) which measures the viability of cells on the basis of their ability to bioreduce 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT). Whether or not MTT reduction occurs within active mitochondria or extramitochrondrially remains the subject of some debate.[38a-e] MTT is yellowish in colour when dissolved in a balanced salt solution without phenol red. In viable cells the tetrazolium ring of MTT is selectively cleaved yielding blue/purple crystals of the formazan derivative. The crystals are dissolved in acidified isopropanol and the resulting colour read spectrophotometrically. A decrease in absorbance when compared to control cells provides a quantitative assessment of cell damage.

Cells were cultured as described above in 96-well plates (Nunc) for 7 days. The growth medium was removed from the cells and they were immediately washed twice with 100 μl HBS-1 (125 mM NaCl; 5 mM KCl; 20 mM $NaHCO_3$; 50 mM HEPES; 5 mM D-glucose; 0.9 mM $CaCl_2$, pH 7.4) per well at 37 °C prior to exposure with 100μl aliquots of test solutions (made up in culture-conditioned medium; final assay concentrations 0.1 - 1000 μM) under the same assay conditions. Following exposure for 24-hr the cells were assayed for cytotoxicity. To assay for cell damage, the cells were washed with 125 μl HBS-1 per well and then 50 μl of MTT solution (final concentration 0.2 mg/ml) was added to each well. The cells were incubated in the dark at 37 °C for 10 min before addition of 125 μl of 10% (w/v) Triton X-100 / 0.04M HCl in anhydrous isopropanol. The plates were then wrapped in aluminium foil and left overnight at 4°C to ensure solubilization of the blue/purple formazan crystals. The absorbance

was read at 570 nm in a Dynatech MR5000 plate reader and cell viability expressed as a percent of control (untreated, 'medium alone' cells).

Functional expression of EAAC1[6]. Transient transfection of exponentially growing HEK293 cells with the EAAC1 encoding construct pCMV-EAAC1 (40 μg/5·10^6 cells) was performed using the calcium phosphate co-precipitation method one day after subculture. At one-to-two days post-transfection, cell cultures were used for electrophysiology.

Whole-cell current recording and solution exchange.[3] Glutamate-induced currents were measured in the whole-cell current-recording configuration at room temperature. The typical resistance of the recording electrode was 2-3 MΩ; the series resistance, 4-6 MΩ. The intracellular solution contained 130 mM KSCN, 1 mM $MgCl_2$, 10 mM TEACl, 10 mM EGTA and 10 mM HEPES (pH 7.3), the extracellular solution contained 140 mM NaCl, 2 mM $MgCl_2$, 2 mM $CaCl_2$ and 10 mM HEPES (pH 7.3). SCN^- was introduced to the intracellular solution because it enhances glutamate transporter-induced currents. For the determination of the electrogenic transport component of the current intracellular SCN^- was replaced by Cl^-. Whole-cell currents were recorded with an Adams & List EPC7 amplifier under voltage-clamp conditions. In general, cells were clamped to 0 mV transmembrane potential.

Glutamate (1 mM) or the new compounds were applied to the cells using a rapid solution exchange device. This device is based on a t-tube mixer design and allows fast switching between 8 different solutions. The velocity of the solution emerging from the porthole of the device (diameter 350 μm) was 5 cm/s and the time resolution was 20-30 ms (10-90 % rise time with whole cells).

Data were low pass-filtered at 0.2 kHz, digitized with a sampling rate of 2 kHz and recorded using the pClamp6 software (Axon Instruments, Foster City, CA). The data were analyzed using the Origin (MicroCal, Northampton, MA) software. Kinetic modeling was performed with the Scientist program (Micromath, Salt Lake City, UT).

Data Evaluation. Fitting of dose-response curves for the evaluation of K_m-values was performed with the Hill-equation $I = I_{max}$ ([S] / ([S] + K_S))^n, where I represents the whole-cell current, [S] the substrate concentration, K_S the apparent substrate affinity, and n the Hill coefficient. A similar equation was used for fitting the outward current dose-response curves generated by the competitive inhibitors in the absence of glutamate. For inhibition of the inward current in the presence of both, inhibitor and glutamate the following fit equation was used:

$$\frac{I - I_0}{I_i - I_0} = \frac{K_i(S)}{[i] + K_i(S)} \qquad (1)$$

In this equation, I_0 represents the current in the presence of only glutamate, I_i is the current in the presence of only the inhibitor of the concentration [i], and I is the current in the presence of inhibitor and glutamate. $K_i(S)$ is the apparent inhibition constant.

Calculation of K_i-values in the presence of glutamate, $K_i(S)$, was performed according to the following equation:

$$K_i(S)/K_i = 1 + [S]/K_S. \qquad (2)$$

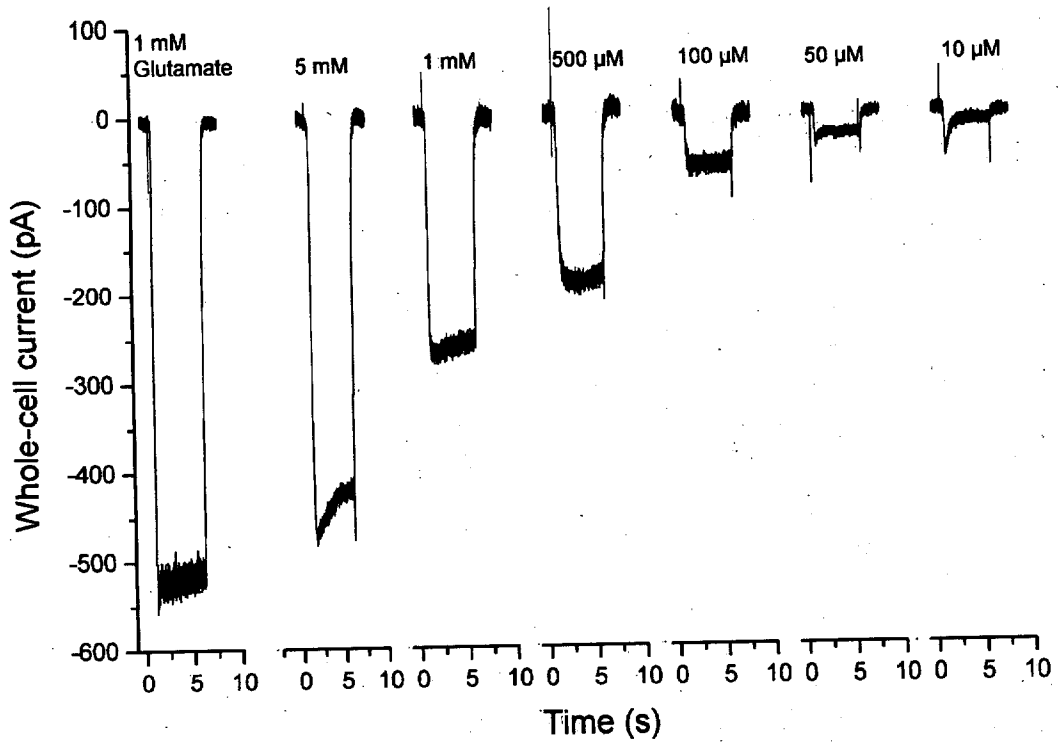

Figure 5A. Determination of Km-values for EAAC1 substrates **1b**: Whole-cell current recording traces (pH 7.4, 22 °C, = mV transmembrane potential, transporter: EAAC1).

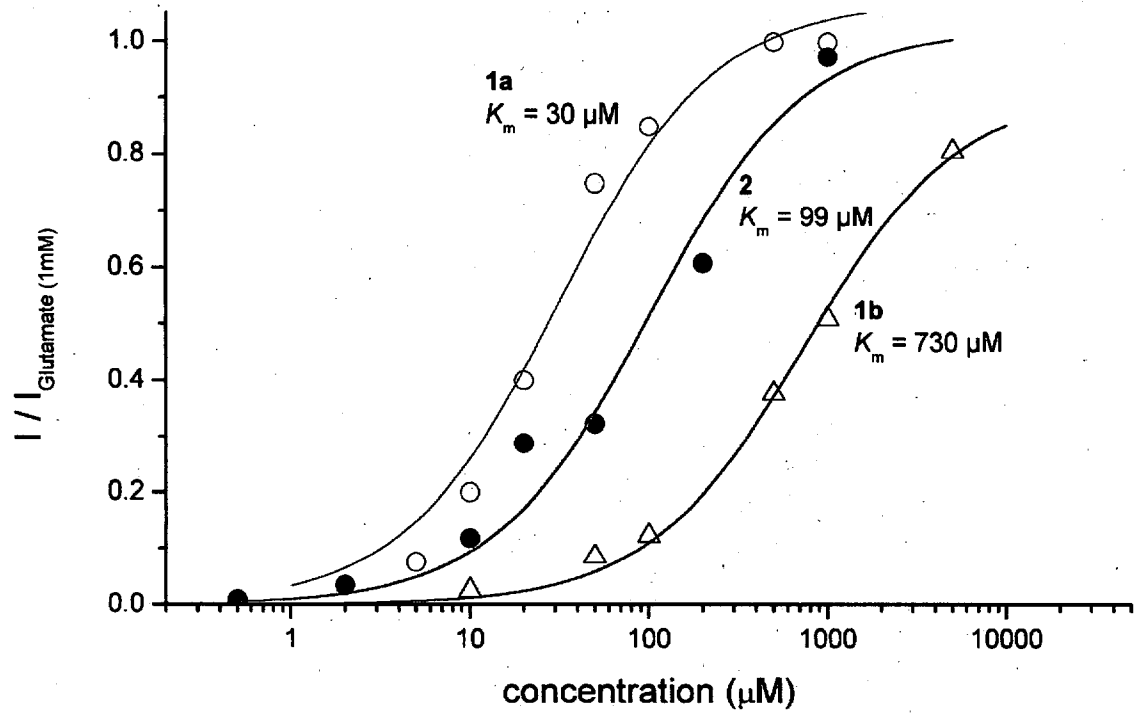

Figure 5B. Determination of K_m-values for EAAC1 substrates **1a,b**, and **2** (pH 7.4, 22 °C, = mV transmembrane potential, transporter: EAAC1).

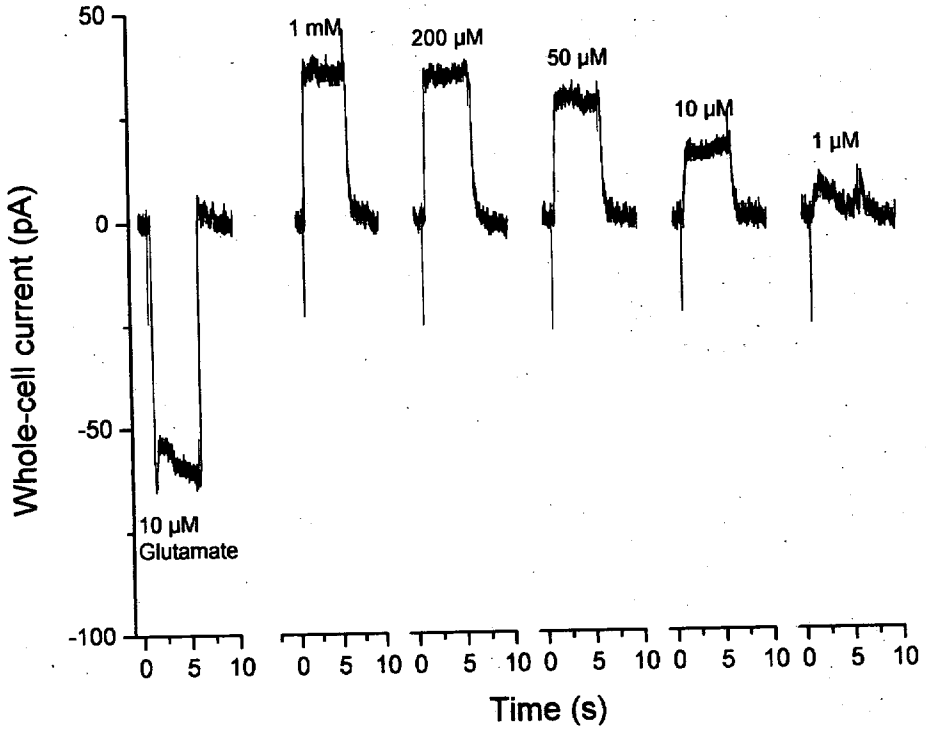

Figure 6. Determination of Km-values for EAAC1 substrates **3**: Whole-cell current recording traces (pH 7.4, 22 °C, = mV transmembrane potential, transporter: EAAC1).

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Table. Elemental Analyses for Compounds **1a,b**, **2-4**, **20a-c**, **21a-c**, **23a,b**, and **24.**

	calcd			found			
compd	C	H	N	C	H	N	formula
1a	41.59	4.99	13.86	41.75	4.82	13.72	$C_7H_{10}N_2O_5$
1b	44.45	5.59	12.96	44.27	5.61	13.10	$C_8H_{12}N_2O_5$
2	44.86	4.71	13.08	45.03	4.49	13.19	$C_8H_{10}N_2O_5$
3	56.17	3.86	5.96	56.39	3.84	6.06	$C_{11}H_9NO_5$
4	56.66	3.03	6.01	56.57	3.23	5.86	$C_{11}H_7NO_5$
20a	54.54	6.10	7.07	54.70	6.12	7.04	$C_{18}H_{24}N_2O_8$
20b	65.16	5.66	5.24	64.96	5.68	5.25	$C_{29}H_{30}N_2O_8$
20c	65.68	5.88	5.11	65.87	5.86	5.12	$C_{30}H_{32}N_2O_8$
21a	57.14	5.86	7.40	57.31	5.84	7.41	$C_{18}H_{22}N_2O_7$
21b	67.43	5.46	5.42	67.56	5.63	5.43	$C_{29}H_{28}N_2O_7$
21c	67.91	5.70	5.28	67.70	5.87	5.11	$C_{30}H_{30}N_2O_7$
23a	72.28	5.09	3.37	72.10	5.10	3.38	$C_{25}H_{21}NO_6$
23b	59.31	4.98	5.32	59.47	4.91	5.16	$C_{13}H_{13}NO_5$
24	59.77	4.24	5.96	59.65	4.15	5.79	$C_{13}H_{11}NO_5$