SUPPORTING INFORMATION

A new phenylalanine derivative acts as an antagonist at the AMPA receptor GluA2 and introduces partial domain closure: Synthesis, resolution, pharmacology and crystal structure

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Experimental methods.

Chemistry. General procedures. All solvents and reagents were purchased from commercial sources and used without further purification. Analytical thin-layer chromatography (TLC) was performed on silica gel 60 F_{254} aluminium plates (Merck). All compounds were detected using UV light and a $KMnO_4$ spraying reagent, the target amino acids were also visualized using a ninhydrin spraying reagent. Flash column chromatography (FC) was performed using CombiFlash Companion System (Teledyne Isco, Inc.) on Redi*Sep* columns with silica gel (average particle size 35 to 70 microns). A mixture of heptane fraction and EtOAc (both HPLC purity) were used as eluents, unless otherwise stated. ^{1}H NMR spectra were recorded on a 300 MHz Varian Mercury 300BB or 300 MHz Varian Gemini 2000BB spectrometer. ^{13}C NMR spectra were recorded on the Varian Gemini spectrometer. $CDCl_3$ (with TMS as internal standard), D_2O and DMSO-d_6 (dimethyl-d_6-sulfoxide) were used as solvents. Chemical shifts (δ) are given in parts per million (ppm), and coupling constants (*J*) are given in Hertz (Hz). The following abbreviations were used: br.s = broad singlet, s = singlet, d = doublet, dd = double doublet, t = triplet, m = multiplet.

2-(4-*tert*-Butylphenyl)acetonitrile (5c). A mixture of 4-*tert*-butylbenzyl bromide **4c** (13.86 g, 61 mmol) and NaCN (4.49 g, 915 mmol) was stirred in dry DMF (60 mL) under a $CaCl_2$ tube at 80 ºC for 6 h. After cooling the solvent was removed under reduced pressure. The resulting yellow oil was then suspended in water (100 mL), pH was adjusted to 10 by adding 1 M NaOH and the mixture was extracted with diethyl ether (3 × 100 mL). The combined organic phases were washed with brine (2 × 100 mL), dried ($MgSO_4$) and evaporated under reduced pressure to afford **5c**. Yield 9.90 g, 94%. The crude product was used in the next step. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.31 (s, 9H, CH_3), 3.71 (s, 2H, CH_2), 7.24–7.29 (m, 4H, Ar).

2-(4-*tert*-Butylphenyl)acetic acid (6c). A mixture of **5c** (9.90 g, 57 mmol) and 60% H_2SO_4 (40 mL) was refluxed for 30 min. The mixture was cooled, diluted with water (50 mL) and extracted with diethyl ether (3 × 100 mL). The ether phases were extracted with water and 0.1 M NaOH (2 × 100 mL). Water washings were treated with 1 M HCl to pH 2 and extracted with diethyl ether (3 × 50 mL). The combined organic phases were dried ($MgSO_4$) and evaporated under reduced pressure to afford **6c** as a yellow oil. Yield 4.12 g, 38%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.31 (s, 9H, CH_3), 3.61 (s, 2H, CH_2), 7.21 (d, *J* = 8.3 Hz, 2H, Ar), 7.35 (d, *J* = 8.3 Hz, 2H, Ar).

2-(4-*tert*-Butylphenyl)ethanol (7c). To a stirred solution of **6c** (5.77 g, 30 mmol) in dry THF (150 mL), a 1 M solution of $LiAlH_4$ in THF (100 mL, 100 mmol) was added dropwise over a period of 1 h keeping the temperature <5 ºC. After addition was completed the mixture was refluxed for 24 h under nitrogen atmosphere. After cooling the mixture was quenched by slow addition of water (50 mL), followed by 15% NaOH (100 mL).

The mixture was stirred at rt for 12 h, then treated with NaCl (10 g), extracted with diethyl ether (3 × 100 mL), dried ($MgSO_4$) and evaporated under reduced pressure to afford **7c** as a yellow oil. Yield 3.68 g, 69%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.31 (s, 9H, CH_3), 2.83 (t, *J* = 6.5 Hz, 2H, CH_2), 3.84 (t, *J* = 6.5 Hz, 2H, CH_2), 7.16 (d, *J* = 7.9 Hz, 2H, Ar), 7.38 (d, *J* = 7.9 Hz, 2H, Ar).

General procedure for the synthesis of isochromans 8b–d. A mixture of the substituted phenylethyl alcohol **7** (64 mmol), (2-methoxyethoxy)methyl (MEM) chloride (11.96 g, 96 mmol) and *N,N*-diisopropylethylamine (12.41 g, 96 mmol) in dry dichloromethane (120 mL) was stirred under nitrogen atmosphere for 2.5 h at rt. The reaction mixture was then washed with 1 M HCl (2 × 120 mL), dried ($MgSO_4$) and the solvent was removed *in vacuo*. The crude MEM acetal was dissolved in dry dichloromethane (40 mL) and added to a cooled (0 ºC) solution of titanium tetrachloride (18.21 g, 96 mmol) in dry dichloromethane (360 mL). The reaction was carried out under nitrogen atmosphere, stirring and keeping the temperature at 0 ºC, for 2.5 h. Then the mixture was quenched by the addition of methanol (15 mL) and 1 M $NaHCO_3$ (100 mL). The organic phase was washed with brine (200 mL), dried ($MgSO_4$) and evaporated under reduced pressure. Purification by FC afforded 7-substituted isochromans **8** as yellow oils.

7-Methylisochroman (8b). Yield 6.74 g, 71%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.34 (s, 3H, CH_3), 2.86 (t, *J* = 5.8 Hz, 2H, CH_2), 3.44 (t, *J* = 5.8 Hz, 2H, CH_2), 4.78 (s, 2H, CH_2), 6.82 (s, 1H, Ar), 6.99–7.06 (m, 2H, Ar); ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.3, 28.1, 65.6, 68.1, 124.9, 127.2, 128.7, 130.1, 134.6, 135.5.

7-*tert*-Butylisochroman (8c). Yield 5.48 g, 45%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.30 (s, 9H, $C(CH_3)_3$), 2.82 (t, *J* = 5.8 Hz, 2H, CH_2), 3.97 (t, *J* = 5.7 Hz, 2H, CH_2), 4.78 (s, 2H, CH_2), 6.99 (br.s, 1H, Ar), 7.06 (d, *J* = 8.1 Hz, 1H, Ar), 7.09 (dd, J_1 = 7.9 Hz, J_2 = 2.1 Hz, 1H, Ar); ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.2, 31.7, 34.7, 65.8, 68.5, 121.2, 123.7, 128.7, 129.4, 130.4, 149.1.

7-Chloroisochroman (8d). Yield 7.77 g, 72%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.78 (t, *J* = 5.5 Hz, 2H, CH_2), 3.92 (t, *J* = 5.5 Hz, 2H, CH_2), 4.69 (s, 2H, CH_2), 6.93 (s, 1H, Ar), 7.00 (d, *J* = 8.0 Hz, 1H, Ar), 7.09 (dd, J_1 = 8.0 Hz, J_2 = 1.9 Hz, 1H, Ar); ^{13}C NMR (75 MHz, $CDCl_3$): δ 27.9, 65.3, 67.6, 124.4, 126.5, 128.4, 130.2, 131.5, 136.6.

General procedure for the synthesis of compounds 9a–d. A mixture of the commercially available isochroman **8a** or the obtained 7-substituted isochroman **8b–d** (40 mmol) and 33% HBr in AcOH (38.4 mL) was heated in a sealed tube at 110 ºC for 18 h. After cooling to rt the mixture was poured into water (200 mL) and extracted with diethyl ether (3 × 200 mL). The organic phase was washed with 1 M NaOH (200 mL) and brine (200 mL), dried ($MgSO_4$) and evaporated *in vacuo*. The residue was purified by FC to afford the dibromides **9**.

1-(2-Bromoethyl)-2-(bromomethyl)benzene (9a). Yield 7.55 g, 68%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.25 (t, *J* = 7.7 Hz, 2H, CH_2), 3.59 (t, *J* = 7.7 Hz, 2H, CH_2), 4.50 (s, 2H, CH_2), 7.17–7.31 (m, 4H, Ar); ^{13}C NMR (75 MHz, $CDCl_3$): δ 31.5, 32.2, 36.0, 128.7, 129.5, 130.3, 131.3, 136.1, 138.0.

1-(2-Bromoethyl)-2-(bromomethyl)-4-methylbenzene (9b). Yield 9.59 g, 82%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.36 (s, 3H, CH_3), 3.29 (t, *J* = 7.7 Hz, 2H, CH_2), 3.65 (t, *J* = 7.7 Hz, 2H, CH_2), 4.55 (s, 2H, CH_2), 7.14–7.19 (m, 3H, Ar); ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.1, 31.7, 32.2, 35.5, 130.0, 130.1, 131.4, 134.7, 135.6, 137.3.

1-(2-Bromoethyl)-2-(bromomethyl)-4-*tert*-butylbenzene (9c). Yield 12.56 g, 94%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.31 (s, 9H, CH_3), 3.27 (t, *J* = 7.8 Hz, 2H, CH_2), 3.63 (t, *J* = 7.8 Hz, 2H, CH_2), 4.56 (s, 2H, CH_2), 7.16 (d, *J* = 7.8 Hz, 1H, Ar), 7.30–7.36 (m, 2H, Ar); ^{13}C NMR (75 MHz, $CDCl_3$): δ 31.5, 32.1, 32.2, 34.8, 35.7, 126.5, 127.9, 130.0, 134.9, 135.4, 150.7.

1-(2-Bromoethyl)-2-(bromomethyl)-4-chlorobenzene (9d). Yield 10.87 g, 87%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.25 (t, *J* = 7.3 Hz, 2H, CH_2), 3.61 (t, *J* = 7.3 Hz, 2H, CH_2), 4.47 (s, 2H, CH_2), 7.17 (d, *J* = 7.9 Hz, 1H, Ar), 7.27 (d, J = 7.9 Hz, 1H, Ar), 7.34 (s, 1H, Ar); ^{13}C NMR (75 MHz, CDCl3): δ 30.2, 31.6, 35.1, 129.3, 130.1, 131.5, 133.1, 136.1, 137.6.

General procedure for the synthesis of compounds 10a–d. A solution of diethyl acetamidomalonate (8.47 g, 39 mmol) in dry dimethylformamide (DMF, 45 mL) was added to a cooled (5 ºC) 60% suspension of NaH in mineral oil (0.94 g, 39 mmol) in dry DMF (60 mL) and the resulting mixture was stirred under nitrogen atmosphere for 30 min. A solution of dibromide **9** (30 mmol) in dry DMF (45 mL) was then added and the mixture was stirred under nitrogen at rt for 16 h. Then the mixture was treated with AcOH (1 mL) and the solvent was removed under reduced pressure. The residue was suspended in water (150 mL) and extracted with dichloromethane (3 × 120 mL), dried ($MgSO_4$) and evaporated *in vacuo*. Purification by FC afforded bromomalonates **10** as white solids.

Diethyl 2-acetamido-2-(2-(2-bromoethyl)benzyl)malonate (10a). Yield 7.21 g, 58%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.21 (t, *J* = 7.2 Hz, 6H, CH_3), 1.94 (s, 3H, CH_3), 3.02 (t, *J* = 8.0 Hz, 2H, CH_2), 3.39 (t, *J* = 8,0 Hz, 2H, CH_2), 3.64 (s, 2H, CH_2), 4.13–4.26 (m, 4H, CH_2), 6.49 (s, 1H, NH), 7.08–7.19 (m, 4H, Ar); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.1, 23.2, 32.3, 34.1, 36.0, 63.2, 67.4, 127.0, 127.8, 130.0, 131.1, 133.8, 138.4, 168.0, 169.9.

Diethyl 2-acetamido-2-(2-(2-bromoethyl)-5-methylbenzyl)malonate (10b). Yield 8.39 g, 65%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.31 (t, *J* = 7.2 Hz, 6H, CH_3), 2.03 (s, 3H, CH_3), 2.27 (s, 3H, CH_3), 3.05 (t, *J* = 7.7 Hz, 2H, CH_2), 3.44 (t, *J* = 7.7 Hz, 2H, CH_2), 3.67 (s, 2H, CH_2), 4.21–4.34 (m, 4H, CH_2), 6.53 (s, 1H, NH), 6.78 (s, 1H, Ar), 7.00–7.07 (m, 2H, Ar); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.2, 21.2, 23.2, 32.7, 34.2, 35.4, 62.9, 67.0, 128.4, 129.6, 131.9, 133.1, 135.4, 136.3, 167.7, 169.2.

Diethyl 2-acetamido-2-(2-(2-bromoethyl)-5-*tert*-butylbenzyl)malonate (10c). Yield 6.07 g, 43%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.27 (s, 9H, CH_3), 1.29 (t, J = 7.1 Hz, 6H, CH_3), 2.00 (s, 3H, CH_3), 3.05 (t, J = 7.9 Hz, 2H, CH_2), 3.45 (t, J = 7.9 Hz, 2H, CH_2), 3.69 (s, 2H, CH_2), 4.20–4.40 (m, 4H, CH_2), 6.54 (s, 1H, NH), 7.00 (d, J = 2.2 Hz, 1H, Ar), 7.09 (d, J = 8.0 Hz, 1H, Ar), 7.23 (dd, J_1 = 8.0 Hz, J_2 = 2.2 Hz, 1H, Ar); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.3, 23.4, 31.7, 32.6, 34.6, 34.7, 35.5, 63.0, 67.2, 124.7, 128.2, 129.4, 132.8, 135.4, 149.6, 167.8, 169.2.

Diethyl 2-acetamido-2-(2-(2-bromoethyl)-5-chlorobenzyl)malonate (10d). Yield 9.56 g, 71%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.29 (t, J = 7.2 Hz, 6H, CH_3), 2.03 (s, 3H, CH_3), 3.04 (t, J = 7.7 Hz, 2H, CH_2), 3.43 (t, J = 7.7 Hz, 2H, CH_2), 3.67 (s, 2H, CH_2), 4.18–4.38 (m, 4H, CH_2), 6.55 (s, 1H, NH), 6.96 (d, J = 2.2 Hz, 1H, Ar), 7.09 (d, J = 8.3 Hz, 1H, Ar), 7.17 (dd, J_1 = 8.3 Hz, J_2 = 2.2 Hz, 1H, Ar); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.1, 23.1, 32.1, 34.0, 34.9, 63.0, 66.9, 127.6, 130.8, 130.9, 132.4, 135.4, 136.8, 167.3, 169.4.

Synthesis of compounds 8f,g. Dichloroisochromans **8f** and **8g** were prepared from 2-(3,4-dichlorophenyl)ethanol (4.20 g, 22 mmol) by the method described for compounds **8a–d**. Purification by FC afforded a mixture of 6,7-dichloro (**8f**) and 7,8-dichloro (**8g**) regioisomers (1.6:1 ratio, determined by proton NMR). The mixture was used in the next step. Small amounts of separated pure compounds **8f** and **8g**, obtained with very low yield after column chromatography were used to confirm the structure. Total yield 3.58 g, 80%.

6,7-Dichloroisochroman (8f). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.81 (t, J = 5.8 Hz, 2H, CH_2), 3.94 (t, J = 5.8 Hz, 2H, CH_2), 4.70 (s, 2H, CH_2), 7.07 (s, 1H, Ar), 7.21 (s, 1H, Ar); ^{13}C NMR (75 MHz, $CDCl_3$): δ 27.5, 64.8, 67.0, 126.0, 127.6, 129.8, 130.3, 133.2, 134.7.

7,8-Dichloroisochroman (8g). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.83 (t, J = 5.8 Hz, 2H, CH_2), 3.92 (t, J = 5.8 Hz, 2H, CH_2), 4.76 (s, 2H, CH_2), 6.98 (d, J = 8.3 Hz, 1H, Ar), 7.28 (d, J = 8.3 Hz, 1H, Ar); ^{13}C NMR (75 MHz, $CDCl_3$): δ 27.8, 64.4, 66.6, 127.8, 128.3, 129.4, 130.0, 133.8, 134.3.

Synthesis of compounds 9f,g. The mixture of dichloroisochromans **8f** and **8g** (3.05 g, 15 mmol) and 33% HBr in AcOH (14.4 mL) was heated in a sealed tube at 110 ºC for 18 h. After cooling to rt the mixture was poured into water (75 mL) and extracted with diethyl ether (3 × 75 mL). The combined organic phases were washed with 1 M NaOH (75 mL) and brine (75 mL), dried ($MgSO_4$) and the solvent was removed *in vacuo*. The residue was purified by FC to afford an almost inseparable mixture of two regioisomeric dibromides **9f** and **9g** in a 2:1 ratio, respectively (determined by proton NMR). The mixture was used in the next step. Total yield 4.76 g, 91%.

1-(2-Bromoethyl)-2-(bromomethyl)-4,5-dichlorobenzene (9f). ^{1}H NMR (300 MHz, CDCl3): δ 3.25 (t, J = 7.4 Hz, 2H, CH2), 3.63 (t, J = 7.4 Hz, 2H, CH2), 4.46 (s, 2H, CH2), 7.34 (s, 1H, Ar), 7.45 (s, 1H, Ar); ^{13}C NMR (75 MHz, $CDCl_3$): δ 29.5, 31.2, 34.8, 129.1, 130.4, 131.9, 132.3, 136.0, 137.6.

1-(2-Bromoethyl)-2-(bromomethyl)-3,4-dichlorobenzene (9g). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.30 (t, *J* = 7.4 Hz, 2H, CH_2), 3.61 (t, *J* = 7.4 Hz, 2H, CH_2), 4.71 (s, 2H, CH_2), 7.11 (d, *J* = 8.5 Hz, 1H, Ar), 7.41 (d, *J* = 8.5 Hz, 1H, Ar); ^{13}C NMR (75 MHz, $CDCl_3$): δ 27.7, 34.9, 36.2, 131.3, 132.5, 133.1, 133.8, 135.8, 138.9.

Synthesis of compounds 10f,g. A solution of diethyl acetamidomalonate (2.82 g, 13 mmol) in dry DMF (15 mL) was added to a cooled (0 ºC) 60% suspension of NaH in mineral oil (312 mg, 13 mmol) in dry DMF (20 mL) and the resulting mixture was stirred under nitrogen atmosphere for 30 min. A solution of dibromides **9f** and **9g** (3.47 g, 10 mmol) in dry DMF (15 mL) was added and the mixture was stirred under nitrogen at rt for 16 h. Then the mixture was treated with AcOH (0.3 mL) and the solvent was removed under reduced pressure. The residue was suspended in water (50 mL) and extracted with dichloromethane (3 × 40 mL), dried ($MgSO_4$) and the solvent was removed *in vacuo*. FC resulted in a mixture of **10f** and **10g**. Total yield 2.78 g, 58%. Pure regioisomeric bromomalonates **10f** (1.21 g) and **10g** (298 mg) were obtained by fractional crystallization (heptane).

Diethyl 2-acetamido-2-(2-(2-bromoethyl)-4,5-dichlorobenzyl)malonate (10f). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.32 (t, *J* = 7.2 Hz, 6H, CH_3), 2.05 (s, 3H, CH_3), 3.05 (t, *J* = 7.4 Hz, 2H, CH_2), 3.46 (t, *J* = 7.4 Hz, 2H, CH_2), 3.67 (s, 2H, CH_2), 4.22–4.35 (m, 4H, CH_2), 6.57 (s, 1H, NH), 7.08 (s, 1H, Ar), 7.26 (s, 1H, Ar); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.2, 23.3, 31.7, 33.7, 34.8, 63.3, 66.8, 130.7, 131.3, 131.5, 132.6, 133.8, 138.6, 167.3, 169.6.

Diethyl 2-acetamido-2-(6-(2-bromoethyl)-2,3-dichlorobenzyl)malonate (10g). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.27 (t, *J* = 7.2 Hz, 6H, CH_3), 1.88 (s, 3H, CH_3), 3.29 (t, *J* = 7.2 Hz, 2H, CH_2), 3.50 (t, *J* = 7.2 Hz, 2H, CH_2), 3.97 (s, 2H, CH_2), 4.17–4.31 (m, 4H, CH_2), 6.64 (s, 1H, NH), 7.06 (d, *J* = 8.5 Hz, 1H, Ar), 7.33 (d, *J* = 8.5 Hz, 1H, Ar); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.1, 22.9, 32.7, 34.3, 36.2, 63.1, 65.7, 128.9, 129.1, 131.7, 134.5, 134.8, 140.3, 167.7, 169.0.

General procedure for the synthesis of cyanomalonates 11a–d,f,g. A mixture of NaCN (147 mg, 3 mmol) and bromomalonate **10** (2.5 mmol) in 10 mL dry DMF (**11a,c,f,g**) or DMSO (**11b,d**) was stirred under a $CaCl_2$ tube at 80 ºC for 2 h. After cooling the mixture was poured into water (25 mL) and extracted with EtOAc (5 × 20 mL). The organic phases were washed with water (3 × 20 mL), brine (20 mL), dried ($MgSO_4$) and the solvent was removed *in vacuo*. The product was purified by FC to obtain **11**.

Diethyl 2-acetamido-2-(2-(2-cyanoethyl)benzyl)malonate (11a). Yield 450 mg, 50%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.28 (t, *J* = 6,8 Hz, 6H, CH_3), 2.01 (s, 3H, CH_3), 2.55 (t, *J* = 6.8 Hz, 2H, CH_2), 2.93 (t, *J* = 6.8 Hz, 2H, CH_2), 3.71 (s, 2H, CH_2), 4.20–4.38 (m, 4H, CH_2), 6.61 (s, 1H, NH), 6.99 (d, *J* = 6.7 Hz, 1H, Ar), 7.14–7.39 (m, 3H, Ar); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.5, 19.4, 23.3, 28.2, 34.3, 63.0, 67.2, 119.0, 127.4, 128.0, 129.7, 131.0, 133.2, 137.9, 167.7, 169.8.

Diethyl 2-acetamido-2-(2-(2-cyanoethyl)-5-methylbenzyl)malonate (11b). Yield 751 mg, 80%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.31 (t, J = 7.2 Hz, 6H, CH_3), 2.02 (s, 3H, CH_3), 2.28 (s, 3H, CH_3), 2.53 (t, J = 7.4 Hz, 2H, CH_2), 2.88 (t, J = 7.4 Hz, 2H, CH_2), 3.66 (s, 2H, CH_2), 4.22–4.34 (m, 4H, CH_2), 6.57 (s, 1H, NH), 6.78 (s, 1H, Ar), 7.02–7.11 (m, 2H, Ar); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.2, 19.5, 21.3, 23.2, 27.6, 34.2, 63.0, 67.2, 119.1, 128.7, 129.4, 131.6, 132.9, 134.7, 136.7, 167.6, 169.4.

Diethyl 2-acetamido-2-(5-*tert*-butyl-2-(2-cyanoethyl)benzyl)malonate (11c). Yield 614 mg, 59%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.27 (s, 9H, CH_3), 1.30 (t, J = 7.2 Hz, 6H, CH_3), 2.00 (s, 3H, CH_3), 2.53 (t, J = 7.3 Hz, 2H, CH_2), 2.87 (t, J = 7.3 Hz, 2H, CH_2), 3.68 (s, 2H, CH_2), 4.20–4.40 (m, 4H, CH_2), 6.57 (s, 1H, NH), 6.99 (d, J = 1.8 Hz, 1H, Ar), 7.14 (d, J = 8.2 Hz, 1H, Ar), 7.25 (dd, J_1 = 8.2 Hz, J_2 = 1.8 Hz, 1H, Ar).

Diethyl 2-acetamido-2-(5-chloro-2-(2-cyanoethyl)benzyl)malonate (11d). Yield 843 mg, 85%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.29 (t, J = 7.2 Hz, 6H, CH_3), 2.01 (s, 3H, CH_3), 2.52 (t, J = 7.2 Hz, 2H, CH_2), 2.87 (t, J = 7.2 Hz, 2H, CH_2), 3.66 (s, 2H, CH_2), 4.18–4.38 (m, 4H, CH_2), 6.59 (s, 1H, NH), 6.96 (d, J = 1.9 Hz, 1H, Ar), 7.14 (d, J = 8.3 Hz, 1H, Ar), 7.19 (dd, J_1 = 8.3 Hz, J_2 = 1.9 Hz, 1H, Ar); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.1, 19.1, 23.1, 27.3, 34.0, 63.1, 66.9, 118.6, 127.9, 130.8, 132.8, 135.2, 136.1, 167.2, 169.6.

Diethyl 2-acetamido-2-(4,5-dichloro-2-(2-cyanoethyl)benzyl)malonate (11f). Yield 140 mg, 13%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.30 (t, J = 7.2 Hz, 6H, CH_3), 2.02 (s, 3H, CH_3), 2.55 (t, J = 7.3 Hz, 2H, CH_2), 2.87 (t, J = 7.2 Hz, 2H, CH_2), 3.66 (s, 2H, CH_2), 4.19–4.36 (m, 4H, CH_2), 6.65 (s, 1H, NH), 7.09 (s, 1H, Ar), 7.31 (s, 1H, Ar); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.3, 19.1, 23.6, 27.4, 33.8, 63.4, 67.0, 118.4, 131.1, 131.2, 131.8, 132.6, 133.8, 138.0, 167.3, 169.8.

Diethyl 2-acetamido-2-(2,3-dichloro-6-(2-cyanoethyl)benzyl)malonate (11g). Yield 301 mg, 28%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.31 (t, J = 7.2 Hz, 6H, CH_3), 1.89 (s, 3H, CH_3), 2.66 (t, J = 7.0 Hz, 2H, CH_2), 3.19 (t, J = 7.3 Hz, 2H, CH_2), 3.98 (s, 2H, CH_2), 4.20–4.37 (m, 4H, CH_2), 6.86 (s, 1H, NH), 7.17 (d, J = 8.3 Hz, 1H, Ar), 7.41 (d, J = 8.3 Hz, 1H, Ar); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.2 19.2, 22.8, 28.9, 34.6, 63.2, 65.6, 119.0, 128.8, 129.5, 131.9, 134.6, 134.9, 139.9, 167.8, 169.2.

Diethyl 2-acetamido-2-(5-(bromomethyl)-2-(2-cyanoethyl)benzyl)malonate (12). A mixture of **11b** (562 mg, 1.5 mmol) and benzoyl peroxide (44 mg, 0.18 mmol) in tetrachloromethane (20 mL) was refluxed for 1 h. NBS (320 mg, 1.8 mmol) was added in 4 adequate amounts each 15 min. After cooling, the mixture was diluted with dichloromethane (30 mL) and washed with water (30 mL), 1 M NaOH (30 mL), brine (30 mL), dried ($MgSO_4$) and the solvent was removed *in vacuo*. Purification with FC afforded **12** as a solid. Yield 370 mg, 54%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.32 (t, J = 7.2 Hz, 6H, CH_3), 2.04 (s, 3H, CH_3), 2.55 (t, J = 7.2 Hz, 2H, CH_2), 2.91 (t, J = 7.2 Hz, 2H, CH_2), 3.70 (s, 2H, CH_2), 4.24–4.37 (m, 4H, CH_2), 4.42 (s, 2H, CH_2), 6.59 (s, 1H, NH),

7.04 (s, 1H, Ar), 7.17–7.27 (m, 2H, Ar); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.2, 19.5, 21.3, 23.2, 27.6, 34.2, 63.0, 67.2, 119.1, 128.7, 129.4, 131.6, 132.9, 134.7, 136.7, 167.6, 169.4.

Diethyl 2-acetamido-2-(2-(3-amino-3-oxopropyl)-5-chloro-4-nitrobenzyl)malonate (13). A mixture of 100% HNO_3 (0.3 mL, 7.5 mmol) and 96% H_2SO_4 (3 mL) was successively added during 1 h at 0 ºC to a solution of **11d** (1.97 g, 5 mmol) in dichloromethane (5 mL). The reaction mixture was stirred for additional 30 min at 0 ºC and for 5 h at rt. The reaction was then quenched with 10% Na_2SO_4 (50 mL) and the mixture was extracted with dichloromethane (3 × 30 mL), dried ($MgSO_4$) and the solvent was removed *in vacuo*. Purification by FC gave the target compound as a solid. Yield 1.45 g, 64%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.29 (t, *J* = 7.2 Hz, 6H, CH_3), 2.04 (s, 3H, CH_3), 2.48 (t, *J* = 7.3 Hz, 2H, CH_2), 2.91 (t, *J* = 7.3 Hz, 2H, CH_2), 3.75 (s, 2H, CH_2), 4.16–4.40 (m, 4H, CH_2), 6.06 (br. s, 2H, NH_2), 6.78 (s, 1H, NH), 7.14 (s, 1H, Ar), 7.77 (s, 1H, Ar); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.1, 23.1, 27.0, 34.0, 36.2, 63.3, 66.6, 124.3, 126.3, 133.8, 140.2, 141.0, 146.2, 167.0, 170.2, 173.6.

Diethyl 2-acetamido-2-(4-amino-2-(3-amino-3-oxopropyl)-5-chlorobenzyl)malonate (14). To a stirred solution of **13** (916 mg, 2 mmol) in ethanol (40 mL) iron powder (2.24 g, 40 mmol), water (10 mL) and 35% HCl (88 μL, 1 mmol) were added. The mixture was refluxed at 95 ºC for 90 min and filtered hot. The filtrate was evaporated and the residue was extracted with EtOAc (3 × 20 mL). The collected organic phases were dried ($MgSO_4$) and evaporated to give **14** as a white solid, which was used without further purification in the next step. Yield 706 mg, 82%. ^{1}H NMR (300MHz, $CDCl_3$): δ 1.30 (t, *J* = 7.2 Hz, 6H, CH_3), 2.03 (s, 3H, CH_3), 2.38 (t, *J* = 6.6 Hz, 2H, CH_2), 2.75 (t, *J* = 6.6 Hz, 2H, CH_2), 3.58 (s, 2H, CH_2), 4.18–4.38 (m, 4H, CH_2), 5.54 (br.s, 1H, NH_2), 6.06 (br.s, 1H, NH_2), 6.75 (s, 1H, NH), 6.79 (s, 1H, Ar), 6.83 (s, 1H, Ar); ^{13}C NMR (75MHz, $CDCl_3$): δ 14.0, 23.0, 27.7, 33.4, 37.2, 62.8, 67.2, 116.5, 116.9, 123.1, 131.0, 140.0, 142.0, 167.5, 169.9, 174.7.

Diethyl 2-acetamido-2-(2-(3-amino-3-oxopropyl)-5-chloro-4-cyanobenzyl)malonate (15). To a mixture of **14** (218 mg, 0.51 mmol), water (6 mL) and 30% HCl (270 μL) kept at –10 ºC, $NaNO_2$ (0.52 mmol, 36 mg) was slowly added. The mixture was stirred for additional 15 min at –10 ºC. In a separate flask a solution of $CuSO_4$ (94 mg, 0.59 mmol) in water (200 μL) was added to a mixture of KCN (65 mg, 1 mmol), water (155 μL) and 25% NH_3 (0.44 mmol, 30 μL). The obtained solution was poured into ice (20 mL) and the diazonium salt was added. pH of the reaction mixture was then adjusted to 8 by addition of 25% NH_3 and the obtained suspension was extracted with EtOAc (3 × 20 mL), dried ($MgSO_4$) and evaporated to afford a brown solid. The product was purified by FC (using heptane/EtOAc/1% AcOH) to obtain **15**. Yield 80 mg, 36%. 1H NMR (300 MHz, $CDCl_3$): δ 1.30 (t, *J* = 7.2 Hz, 6H, CH_3), 2.04 (s, 3H, CH_3), 2.45 (t, *J* = 8.0 Hz, 2H, CH_2), 2.90 (t, *J* = 8.0 Hz, 2H, CH_2), 3.76 (s, 2H, CH_2), 4.20–4.39 (m, 4H, CH_2), 5.68 (br. s, 2H, $CONH_2$), 6.68 (s, 1H, NH), 7.11 (s, 1H, Ar), 7.48 (s, 1H, Ar).

In vitro Pharmacology.

Native AMPA receptor binding assays. Analogues **3a–j** were evaluated for binding affinity to native rat brain synaptosomal membranes prepared according to the method described by Ransom and Stec.[1] IC_{50} for AMPA receptor sites[2] was determined using 5 nM [^{3}H]AMPA, with some modifications as previously described.[3]

Recombinant rat AMPA receptor binding assays. GluA1$_o$, GluA2(R)$_o$, GluA3$_o$ and GluA4$_o$ were inserted into recombinant baculoviruses, receptors expressed by infection of *Sf9* insect cells and infected *Sf9* cell membranes utilized for radioligand binding assays. GluA1$_o$–4$_o$ were assayed using [^{3}H]-AMPA as previously detailed.[3,4] The mutant (Y716F)GluA1$_o$ was prepared and characterized previously.[5] Bound and free radiolabel were separated by cold filtration through GF/B glass fiber filters (Whatman, Kent UK) using two washes with cold assay buffer. The soluble constructs GluA2-S1S2J and (Y702F)GluA2-S1S2J were prepared and assayed as formerly described.[5] Non-specific binding was determined in the presence of 1 mM (*S*)-Glu. Competition studies were performed using ~5 nM radiolabel in the presence of 10 nM–1 mM cold ligand. Competition data were analyzed using Grafit v3.00 (Erithacus Software Ltd., Horley, UK) as previously detailed.[6]

TEVC Pharmacology. Homomeric GluA2(Q)$_i$ and GluA3$_i$ were expressed in *Xenopus laevis* oocytes and channel activity measured as previously described.[6] Concentration-response curves of **3h** inhibition of (*S*)-Glu responses were fit to a logistic equation to determine slope (n_H) and IC_{50}, from which K_i was calculated using the Cheng-Prusoff equation. K_b values were measured by performing (*S*)-Glu concentration-response curves in the absence and presence of a fixed concentration of **3h**, on the same oocyte. Curves were fit to the logistic equation, equiactive (*S*)-Glu concentrations calculated and analyzed as per Gaddum[7] to extract K_b.

X-ray structure determination.

The GluA2-S1S2J construct developed by Armstrong and Gouaux[8] was used, and the protein was expressed, refolded and purified essentially as reported.[9,10]

GluA2-S1S2J in complex with (*RS*)-**3h** was crystallized by the hanging drop vapor diffusion method at 7 °C. The protein complex solution contained 6.2 mg/mL GluA2-S1S2J and 3.3 mM (*RS*)-**3h** in 10 mM Hepes pH 7.0, 20 mM sodium chloride and 1 mM EDTA. Crystals were obtained in drops consisting of 1 μL complex solution and 1 μL reservoir solution of 0.1 M ammonium sulfate, 0.1 M sodium acetate buffer pH 5.5 and 15.2% PEG 4000. The reservoir volume was 0.5 mL. The crystals grew within one week to a maximum dimension of 0.1 mm.

Crystals of GluA2-S1S2J in complex with (*S*)-**3h** were flash-cooled using ca. 20% glycerol added to the reservoir solution as a cryo-protectant. Complete synchrotron data were collected at 100 K at the beamline ID14-3, ESRF, Grenoble, France equipped with an ADSC Quantum 4 detector and at a wavelength of 0.9310 Å. A full

data set was collected to 1.90 Å resolution. Diffraction data were processed with the programs MOSFLM[11] and SCALA[12] implemented in the program package CCP4i.[13] For crystal data and data collection statistics, see Supplementary Table 1.

The structure was solved by molecular replacement, using the program PHASER[14] within CCP4i. The structure of GluA2-S1S2J in complex with (*S*)-ATPO (PDB ID 1N0T, molA[15]) was used as search model for phasing the data, including protein atoms only. A clear solution comprising two molecules (molA and molB) was obtained. Subsequently, the amino acid residues were traced using ARP/wARP[16] except for a few amino acids, which were manually built using program COOT.[17] Afterwards, (*S*)-**3h** were unambiguously fitted into the electron densities within the ligand binding site of molA and molB, respectively. The structure was further subjected to refinements in REFMAC5[18] within CCP4i and in PHENIX.[19] Between each refinement step, the structure was inspected and corrected using the program COOT. Gradually, water molecules and sulfate ions were added to the structure.

The programme DynDom[20] was employed for analysis of ligand-induced domain closure. Figures were prepared with Pymol.[21] Library files for refinement of ligands were obtained using the PRODRG server.[22]

Table 1. Crystal data, data collection and refinement statistics.

Crystal data	
Space group	$P2_1$
Unit cell parameters (Å, degree)	a = 48.7, b = 61.8, c = 92.9, β = 94.1
Molecules/a.u.[a]	2
Data collection	
Resolution range (Å)	46.3 – 1.90
Unique reflections	41,312
Average redundancy	3.7 (3.5)[b]
Completeness (%)	94.9 (78.6)
R_{sym} (%)[c]	5.7 (25.3)
$I/\sigma(I)$	17.0 (3.9)
Refinement	
Non-hydrogen atoms	4,790
Amino acid residues	519
(*S*)-**3h**	2
Water molecules/sulfate ions	632/4
R_{work}[d] (%)	17.2
R_{free}[e] (%)	23.0
R.m.s.d. bond lengths (Å)/angles (degrees)	0.005/0.95
Residues in allowed regions of Ramachandran plot[f] (%)	99.3
Average B-values (Å^2) for:	
Protein atoms (molA/B)	24/26
(*S*)-**3h** (molA/B)	29/39
Water molecules/sulfate ions	34/79

Footnotes:

a. A.u.: asymmetric unit of the crystal.

b. Numbers in parentheses are for the outermost bin: 2.00 – 1.90 Å.

c. $R_{sym}(I) = \Sigma_{hkl}|I_{hkl}-<I_{hkl}>|/\Sigma_{hkl}I_{hkl.}$

d. $R = \Sigma_{hkl}||F_o|-|F_c||/\Sigma|F_o|$, where $|F_o|$ and $|F_c|$ are observed and calculated structure factor amplitudes, respectively, for reflection *hkl.*

e. 5% of the reflections were set aside for calculation of the R_{free} value.

f. The Ramachandran plot was calculated according to Kleywegt and Jones.[23]

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