Supporting Information

1*H*-Cyclopentapyrimidine-2,4(1*H*,3*H*)-dione-related Ionotropic Glutamate Receptors Ligands. Structure-activity Relationships and Identification of Potent and Selective iGluR5 Modulators

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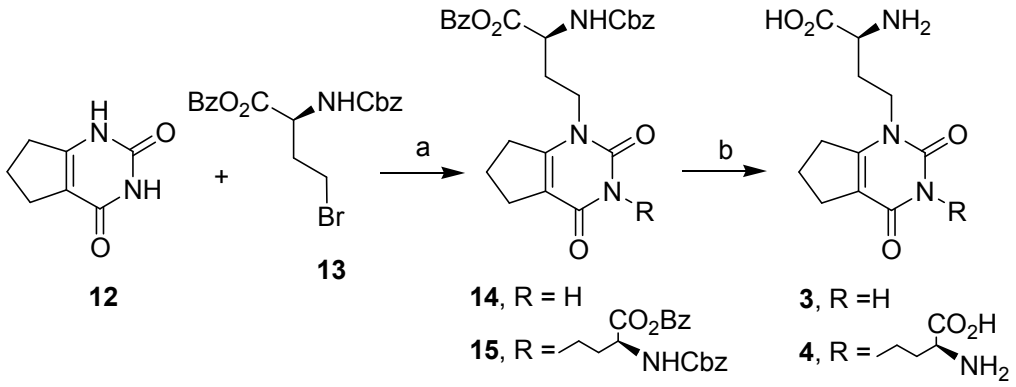
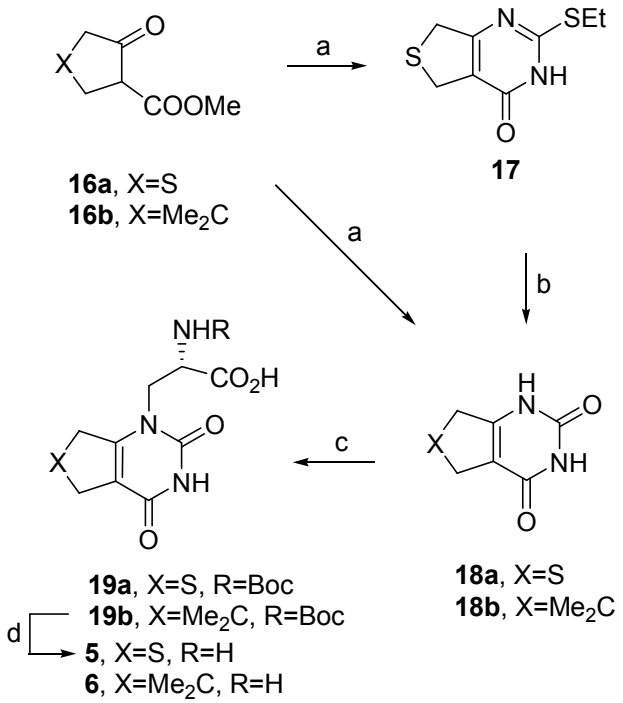
Chemistry, Schemes 1-5, experimental procedures for intermediates, table with elemental analyses for final compounds.

Scheme 1. Synthesis of Compounds **3** and **4**



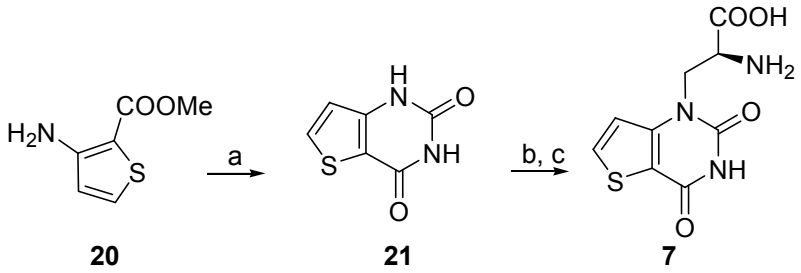
Reagents and conditions: (a) NaH, DMF, -65 °C to rt, 48 h; (b) Pd/C, H_2 (40/90 psi), MeOH, 8/96 h, rt.

Scheme 2. Synthesis of Compounds **5** and **6**



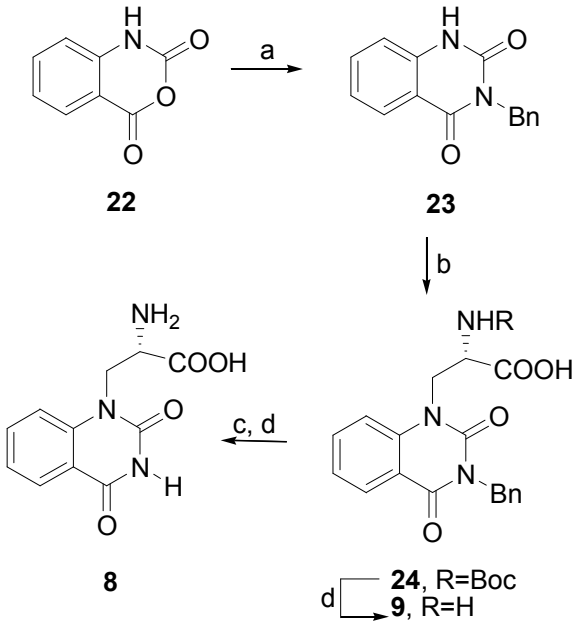
Reagents and conditions: (a) (*S*)-ethylisothiouronium bromide, Na_2CO_3, H_2O, 18 h, rt; b) HCl, AcOH, H_2O, 5 h, reflux; c) NaH, DMF, (*S*)-3-[(*tert*-butoxycarbonyl)amino]oxetan-2-one, -65 °C to rt, 18 h; d) TFA, CH_2Cl_2, 16 h, rt.

Scheme 3. Synthesis of Compound **7**



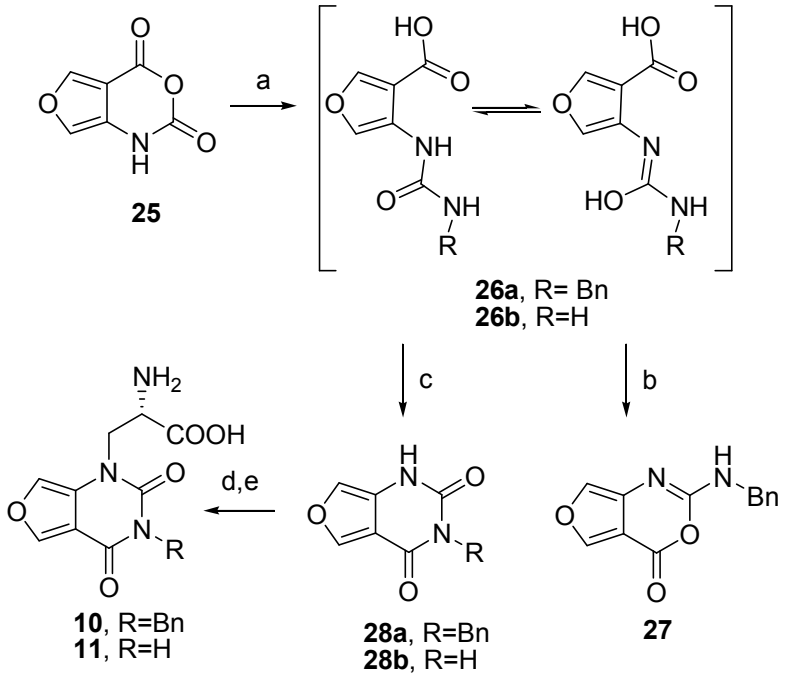
Reagents and conditions: (a) NaOCN, H_2O, AcOH, 5 h, rt; (b) NaH, DMF, (*S*)-3-[(*tert*-butoxycarbonyl)amino]oxetan-2-one, -65 °C to rt, 18 h; (c) TFA, CH_2Cl_2, 16 h, rt

Scheme 4. Synthesis of Compounds **8** and **9**



Reagents and conditions: (a) $BnNH_2$, Urea, DMAC, 160 °C, MW, 250 W, 5 min; (b) NaH, DMF, (*S*)-3-[(*tert*-butoxycarbonyl)amino]oxetan-2-one, -65 °C to rt, 18 h; (c) $HCOONH_4$, Pd/C 10%, MeOH, reflux, 24 h; (d) TFA, CH_2Cl_2, 16 h, rt.

Scheme 5. Synthesis of Compounds **10** and **11**



Reagents and conditions: (a) $BnNH_2$, (for **26a**) or NH_3OH (for **26b**) THF, 48 h, rt (b) CDI, molecular sieves, THF, 3 h reflux; (c) CDI, molecular sieves, dioxane, 3 h reflux; (d) NaH, DMF, (*S*)-3-[(*tert*-butoxycarbonyl)amino]oxetan-2-one, -65 °C to rt, 18 h; (e) TFA, CH_2Cl_2, 16 h, rt.

Chemistry

The new analogues structurally related to (*S*)-**1** are reported in Chart 1 of the main text. Compound (*R*)-**1** was prepared starting from (*R*)-3-[*t*-(butoxycarbonyl)amino]oxetan-2-one following the same synthetic pathway previously reported for its enantiomer (*S*)-**1**.[1] The homo-alanine analogues **3** and **4** were synthesized as reported in Scheme 1. The key intermediate for the synthesis of these two target derivatives is the pyrimidinedione **12**, prepared as previously described.[1] Derivative **12** was then *N*-alkylated using bromoderivative **13**[2]to give both mono-substituted (**14**) and di-substituted (**15**) compounds. Deprotection of **14** and **15** provided **3** and **4** respectively.

To evaluate the influence of a heteroatom and/or a steric hindrance in a spectrum of activity and selectivity among the subunits iGluR1-6, derivatives **5** and **6** were prepared (Scheme 2). The intermediates **16a**[3] and **16b**[4] by reaction with *S*-ethylisothiouronium bromide were transformed into **18a,b**; while **18b** was directly formed, in the case of sulfur heterosubstituted compound, intermediate **17** was isolated and characterized, and acidic cleavage was necessary to obtain **18a**. The *N*-alkylation of **18a,b,** performed using (*S*)-3-[(*tert*-butoxycarbonyl)amino]oxetan-2-one,[5] afforded compounds **19a,b** which, after deprotection, led us to the desired analogues **5** and **6**.

The cyclopentyl ring of (*S*)-**1** was also replaced by different aromatic or heteroaromatic cyclic systems (**7**-**11**) in order to explore the effect of the aromatization and consequent planarization of the skeleton on activity. For the synthesis of derivative **7** (Scheme 3) the key intermediate **21** was reacted with sodium cyanate affording **21** in 87% yield.[6] *N*-Alkylation of **21** by the previously described standard procedure, followed by TFA-mediated deprotection, gave the analogue **7**.

In Scheme 4 is reported the synthetic strategy to obtain compounds **8** and **9**. To orient the synthesis towards a selective monoalkylation at N^1, the initial synthetic strategy was slightly modified. Derivatives **8** and **9** were prepared starting from isatoic anhydride **22** which was transformed into the derivative **23** in the presence of benzylamine under microwave irradiation.[7]

N-Alkylation of compound **23**, afforded derivative **24**. Deprotection of the amino group led to compound **9**, while derivative **8** was obtained from compound **24** by hydrogenolysis of the benzyl function and removal of the *N*-Boc group.[8]

For the synthesis of derivatives **10** and **11** (Scheme 5) the key intermediates were the pyrimidinediones **28a,b**. In a first attempt, we tried to obtain these intermediates from compound **25**[9] following the synthetic approach previously reported in Scheme 4, but this strategy did not provide the desired compound. Therefore, we tried to synthesize **28a**,**b** from derivatives **26a**,**b** obtained by treating compound **25** with benzylamine or ammonium hydroxide.[9] Since intermediates **26a**,**b** were in equilibrium between ureidic and enolic form, we observed in the next step the formation two different isomers (**27** and **28a**,**b**). The selective formation of the oxazine **27** or the pyrimidinediones **28a**,**b** was addressed by using a different solvent that could stabilize the intermediate **26** into the ureidic form or its enolic tautomer. Performing the dehydration reaction in THF we obtained compound **27** as major isomer (30% yield); in contrast after performing the reaction in dioxane, compounds **28a**,**b** were the main products (52% and 18% yield respectively). The final compounds **10** and **11** were obtained from derivatives **28a,b** by the usual *N*-alkylation reaction followed by deprotection of the amino group.

Experimental Procedures

Reagents were purchased from Aldrich and were used as received. Reaction progress was monitored by TLC using Merck silica gel 60 F_{254} (0.040-0.063 mm) with detection by UV. Merck silica gel 60 (0.040-0.063 mm) was used for column chromatography. Melting points were determined in Pyrex capillary tubes using an Electrothermal 8103 apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on Brüker 200 MHz or Varian 300 MHz spectrometer with TMS as internal standard. Splitting patterns are described as singlet (s), doublet (d), triplet (t), quartet (q), and broad (br); the value of chemical shifts (δ) are given in ppm and coupling constants (*J*) in Hertz (Hz). Number of overlapping carbons are reported in

brackets. GC-MS were performed on a Saturn 3 (Varian) or Saturn 2000 (Varian) GC-MS System using a Chrompack DB5 capillary column (30 m x 0.25 mm i. d.; 0.25 μm film thickness). ESI-MS spectra were performed by an Agilent 1100 Series LC/MSD spectrometer and by LCQDeca-THERMOFINNIGAN spectrometer. GC-MS were performed on a Saturn 3 (Varian) or Saturn 2000 (Varian) GC-MS System using a Chrompack DB5 capillary column (30 m x 0.25 mm i. d.; 0.25 μm film thickness). Reaction performed using microwave irradiation were performed by a CEM Discover microwave apparatus. Optical rotations were measured using a Perkin-Elmer 343 polarimeter. Elemental analyses were performed in a Perkin-Elmer 240C elemental analyzer and the results were within ± 0.4% of the theoretical values, unless otherwise noted. Yields refer to purified products and are not optimized. All moisture-sensitive reactions were performed under argon atmosphere using oven-dried glassware and anhydrous solvents.

For testing, compounds (*R*)-**1**,**3-11** were transformed into the corresponding hydrochloride salts by a standard procedure.

(*S*)-1-[3'-(Benzyloxycarbonyl)amino-3'-(benzyloxycarbonyl)propyl]-6,7-dihydro-1*H*-cyclopenta[*d*]pyrimidin-2,4(3*H*,5*H*)-dione (**14**).

(*S*,*S*)-1,3-di[3'-(Benzyloxycarbonyl)amino-3'-(benzyloxycarbonyl)propyl]-6,7-dihydro-1*H*-cyclopenta[*d*]pyrimidin-2,4(3*H*,5*H*)-dione (**15**).

To a suspension of **12** (0.75 g, 4.93 mmol) in freshly distilled *N*,*N*-dimethylformamide (DMF) (55.0 mL), sodium hydride (NaH) (130.0 mg, 5.42 mmol) was added and the mixture was stirred for 2 h at room temperature. Then the mixture was cooled to -65 °C and a solution of **13** (2.00 g, 4.93 mmol) in dry DMF (27.0 mL) was added drop wise during 1 hour. When the addition was complete the mixture was allowed to warm to 25 °C and was stirred for 48 h. The solvent was removed under vacuum, water was added to the residue, the aqueous phase acidified until pH 2 and extracted with EtOAc (3 x 40 mL). The collected organic layers were dried on Na_2SO_4 and then the solvent removed under vacuum. Purification of crude mixture by means of flash

chromatography (33% petroleum ether 40-60 °C in EtOAc) afforded compound **14** as a colorless oil in 15.0% yield and compound **15** as a white amorphous solid in 46.4% yield;

Compound **14**: ^{1}H NMR (200 MHz, $CDCl_3$) δ 9.64 (br, 1H), 7.30 (m, 10H), 6.02 (br, 1H), 5.10-5.03 (m, 4H), 4.53 (m, 1H), 4.02 (t, 2H, *J* = 6.7 Hz), 2.63 (m, 4H), 2.26-1.93 (m, 4H); ESI-MS *m/z* 500 $(M+Na)^+$; $[\alpha]^{20}_D$ = -11.4 (*c* 0.3, $CHCl_3$);

Compound **15**: ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.28 (m, 20H), 6.20 (br, 2H), 5.06 (m, 8H), 4.44 (m, 2H), 4.07 (m, 2H), 3.72 (m, 2H), 2.57 (m, 4H), 2.14 (m, 4H), 1.87 (m, 2H); ESI-MS *m/z* 825 $(M+Na)^+$, 803 $(M+H)^+$; $[\alpha]^{20}_D$ = -8.2 (*c* 0.5, $CHCl_3$).

2-(Ethylthio)thieno[3,4-*d*]pyrimidin-4(3*H*,5*H*,7*H*)-one (17). To a solution of *S*-ethyl-isothiouronium bromide (2.30 g, 12.5 mmol) in water (10.0 mL) kept in the dark, sodium carbonate (Na_2CO_3) (1.30 g, 12.5 mmol) and then **16a** (2.00 g, 12.5 mmol) were added portion wise. The resulting mixture was stirred for 18 h at 25 °C in the dark. The suspension was filtered and the solid was washed with water, diethyl ether, methanol and acetone. Then the collected solid was dried in an oven at 50 °C under vacuum. Crystallization of the solid compound from methanol afforded derivative **17** as white prisms in 84.6% yield: mp (methanol) >300 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 7.90 (br, 1H), 4.06 (m, 2H), 3.87 (m, 2H), 3.06 (q, 2H, *J* = 7.4 Hz), 1.25 (t, 3H, *J* = 7.4 Hz); ^{13}C NMR (200 MHz, DMSO-d_6) δ 165.3, 161.0, 118.3, 114.5, 34.7, 32.0, 24.9, 15.0; ESI-MS *m/z* 237 $(M+Na)^+$.

Thieno[3,4-*d*]pyrimidin-2,4(1*H*,3*H*,5*H*,7*H*)-dione (18a). To a solution of **17a** (2.00 g, 9.34 mmol) in water (15.0 mL), concentrated HCl (1.45 mL) and glacial acetic acid (2.90 mL) were added and the mixture was heated under reflux for 5 h. The resulting suspension was filtered and the solid was washed with water and methanol. The collected solid was dried in an oven at 50 °C under vacuum. Crystallization of the solid compound from methanol afforded derivative **18a** as white prisms in 87.0% yield: mp (methanol) >300 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 11.16 (br, 1H), 11.00 (br, 1H), 3.93 (t, 2H, *J* = 3.3 Hz), 3.72 (t, 2H, *J* = 3.3 Hz); ^{13}C NMR (200

MHz, DMSO-d_6) δ 161.9, 152.8, 152.3, 109.6, 36.0, 32.8; ESI-MS *m/z* 339 $(2M-H)^-$, 169 (100) $(M-H)^-$.

6,6-Dimethyl-1,5,6,7-tetrahydrocyclopentapyrimidin-2,4-dione (18b). To a solution of *S*-ethylisothiouronium bromide (1.15 g, 6.3 mmol) in water (5.0 mL) kept in the dark, Na_2CO_3 (668 mg, 6.3 mmol) and then **16b** (1.07 g, 6.3 mmol) were added portion wise. The resulting mixture was stirred for 18 h at room temperature in the dark. The suspension was filtered and the solid was washed with water, diethyl ether, methanol and acetone. Then the collected solid was dried in an oven at 50 °C under vacuum. Crystallization of the solid compound from methanol afforded derivative **18b** as white solid in 23.0% (mp 290-295 °C)**.** ^{1}H-NMR (200 MHz, DMSO-d_6) δ 10.94 (br, 1H), 10.67 (br, 1H), 2.40 (s, 2H), 2.22 (s, 2H), 1.08 (s, 3H), 1.07 (s, 3H); ESI-MS *m/z* 179 $(M-H)^-$.

(*S*)-1-[2'-(*tert*-Butoxycarbonyl)amino-2'-carboxyethyl]-5,7-dihydrothieno[3,4-*d*]pyrimidin-2,4(1*H*,3*H*)-dione (19a). To a suspension of **18a** (2.0 g, 11.6 mmol) in dry DMF (100.0 mL) NaH (253.0 mg, 10.54 mmol) was added portion wise and the resulting mixture was stirred at 25 °C for 2 h. After the mixture was cooled at -65 °C a solution of (*S*)-3-[(*tert*-butoxycarbonyl)amino]oxetan-2-one (1.97 g, 10.54 mmol) in dry DMF (30.0 mL) was added during 1 hour. When the addition was complete the mixture was allowed to warm to room temperature and left 18 h under stirring. The solvent was removed under reduced pressure, and the crude product taken up in water (30.0 mL). The aqueous phase was acidified until pH 2 and then extracted with ethyl acetate (EtOAc) (3 x 35 mL). The collected organic layers were dried on Na_2SO_4 and then the solvent removed under vacuum. The crude product was purified by means of flash chromatography using a gradient of elution (from CH_2Cl_2/MeOH/AcOH 97:3:0.1 v/v to CH_2Cl_2/MeOH/AcOH 90:20:1 v/v) affording compound **19a** as an amorphous white solid (18.3% yield). ^{1}H NMR (200 MHz, DMSO-d_6) δ 11.22 (br, 1H), 6.66 (br , 1H), 4.21 (m, 4H), 3.75 (m, 2H), 3.43 (m, 1H), 1.28 (s, 9H); ^{13}C NMR (200 MHz, DMSO-d_6) δ 171.7, 160.9, 155.9,

154.2 152.3, 110.8, 79.1, 51.8, 48.8, 36.6, 33.4, 28.6; ESI-MS *m/z* 356 $(M-H)^-$, 282; ESI-MS/MS of $(M-H)^-$ *m/z* 282 (100), 238; $[\alpha]^{20}_D$ = -52 (*c* 0.3, 1N HCl).

(*S*)-1-[2'-(*tert*-Butoxycarbonyl)amino-2'-carboxyethyl]-6,6-dimethyl-2,3,4,5,6,7-hexahydrocyclopentapyrimidin-2,4-dione (**19b**) The title compound was synthesized following the synthetic strategy previously reported for compound **19a**. **19b** was obtained as an amorphous solid in 44% yield. ^{1}H NMR (200 MHz, CD_3OD) δ 4.60-4.23 (m, 2H), 3.76 (m, 1H), 2.75 (m, 2H), 2.42 (s, 2H), 1.36 (s, 9H), 1.19 (m, 6H); ESI-MS *m/z* 366 $(M-H)^-$, 292; $[\alpha]^{20}_D$ = -87 (*c* 0.6, 1N HCl).

Thieno[3,2-*d*]pyrimidin-2,4-dione (21). A solution of sodium cyanate (5.00 g, 77.0 mmol) in water (15.0 mL) was added drop wise to a solution of **20** (6.05 g, 38.4 mmol) in 50% of glacial acetic acid in water (90.0 mL) and the resulting mixture was stirred for 5 h at room temperature. The resulting precipitate was collected by filtration and then dissolved in NaOH 2N (90.0 mL). The solution was cooled to 0 °C, acidified with HCl 4N and the solid filtered and dried in an oven at 60 °C to give **21** (87% yield) as a white solid: mp >300 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 9.17 (br, 1H), 7.91 (d, 1H, *J* = 5.2 Hz), 7.72 (d, 1H, *J* = 5.2 Hz), 6.70 (br, 1H); ESI-MS *m/z* 191 $(M+Na)^+$.

3-Benzylquinazoline-2,4(1*H*,3*H*)-dione (23). A round bottom flask containing a mixture of **22** (400.0 mg, 2.45 mmol), benzylamine (0.33 mL, 3.00 mmol), urea (150.0 mg, 2.45 mmol) and *N*,*N*-dimethylacetamide (DMAC) (2.0 mL) was irradiated in a microwave oven at 250 W for 5 at 160 °C After cooling to room temperature water was added to the mixture and the resulting solid was filtered. The crude product was purified by means of flash chromatography (EtOAc) to obtain **23** as a white solid (91.2% yield): mp (acetone) 223-224 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 11.46 (br, 1H), 7.90 (d, 1H, *J* = 7.8 Hz), 7.63 (m, 1H), 7.28-7.14 (m, 7H), 5.05 (s, 2H); ESI-MS *m/z* 251 $(M-H)^-$.

(*S*)-1-[2'-(*tert*-Butoxycarbonyl)amino-2'-carboxyethyl]-3-benzylquinazoline-2,4(1*H*,3*H*)-dione (24). The title compound was prepared following the above described procedure for **19a**

starting from **23** (250.0 mg, 1.00 mmol). Compound **24** was obtained as a colorless oil in 49.7% yield. ^{1}H NMR (200 MHz, CD_3OD) δ 8.06 (d, 1H, *J* = 7.6 Hz), 7.65 (m, 2H), 7.34 (m, 2H), 7.18 (m, 4H), 5.17 (m, 2H), 4.57-4.43 (m, 3H), 1.10 (s, 9H); ESI-MS *m/z* 438 $(M-H)^-$, 364; $[\alpha]^{20}_D$ = +4.8 (c 1.0, MeOH).

2-(Benzylamino)-4*H*-furo[3,4-*d*][1,3]oxazin-4-one (27). Benzylamine (46.3 µL, 0.42 mmol) was added to a suspension of **25** (50.0 mg, 0.33 mmol) in dry tetrahydrofuran (2.5 mL) and the mixture was stirred at 25 °C for 48 h. Then the solvent was removed and the crude intermediate was re-dissolved in dry tetrahydrofuran (2.0 mL). To this solution 1,1'-carbonyldiimidazole (CDI) (68.1 mg, 0.42 mmol) and activated molecular sieves were added. The mixture was refluxed for 3 h. The solvent was removed in vacuum and the crude product purified by flash chromatography (EtOAc) to afford **27** as an amorphous solid (30% yield). ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.05 (s, 1H), 7.79 (br, 1H), 7.42 (s, 1H), 7.30 (m, 4H), 6.86 (s, 1H), 4.53 (d, 2H, *J* = 5.6 Hz); ^{13}C NMR (300 MHz, acetone-*d6*) δ 149.4, 138.7 (2C), 136.1, 130.1, 128.7, 127.9, 127.5 (2C), 116.5, 44.3; ESI-MS *m/z* 241 $(M-H)^-$; GC-MS *m/z* 133, 104, 91 (100), 77, 65.

3-Benzylfuro[3,4-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (28a). To a suspension of **25** (50.0 mg, 0.33 mmol) in dry dioxane (2.5 mL), benzylamine (46.3 µL, 0.42 mmol) was added and the mixture stirred at 25 °C for 48 h. After that, the solvent was removed and the crude intermediate was re-dissolved in dry dioxane (2.0 mL). To this solution 1,1'-carbonyldiimidazole (CDI) (68.1 mg, 0.42 mmol) and activated molecular sieves were added and the reaction mixture was refluxed for 3 h under stirring. The solvent was removed under vacuum and the crude product purified by means of flash chromatography (EtOAc) to afford **28a** as a colorless oil (52% yield). ^{1}H NMR (200 MHz, acetone-*d6*) δ 9.81 (br, 1H), 8.24 (d, 1H, *J* = 1.5 Hz), 7.55 (d, 1H, *J* = 1.5 Hz), 7.39-7.20 (m, 5H), 5.07 (s, 2H); ESI-MS *m/z* 241 $(M-H)^-$, ESI-MS/MS of $(M-H)^-$ 108.

Furo[3,4-*d*]pyrimidin-2,4(1*H*,3*H*)-dione (28b). The title compound was synthesized starting from **25** and ammonium hydroxide, and following the synthetic strategy previously reported for compound **28a**. Purification by means of flash chromatography (EtOAc/MeOH 10:1) afforded

28b as a pale yellow amorphous solid (18% yield). ^{1}H-NMR (200 MHz, DMSO-*d6*) δ 10.77 (br, 1H); 10.51 (br, 1H), 8.33 (s, 1H), 7.52 (s, 1H); ESI-MS *m/z* 151 $(M-H)^-$.

In Vitro Pharmacology.

Recombinant Baculovirus and *Sf9* Cell Culture. The baculovirus-*Sf9* insect cell system was employed to express recombinant rat iGluR complexes used for radioligand binding assays. All manipulations of baculovirus and insect cells were according to standard protocols in 'Guide to Baculovirus Expression Vector Systems and Insect Cell Culture Techniques', (Life Techologies, Paisley, UK) and 'Baculovirus Expression Vector System: Procedures and Methods Manual', 2nd ed. (PharMingen, San Diego, CA). The creation and expression of the recombinant iGluR baculoviruses has been described previously.[10]

Radioligand Binding. The affinities of compounds at the recombinant rat AMPA-R ($iGluR1_o$, $iGluR2(R)_o$, $iGluR3_o$, $iGluR4_o$) and KA-R [$iGluR5(Q)_{1b}$ and iGluR6(*V,C,R*)], expressed homomerically in *Sf9* cell membranes, were determined from competition experiments with: (*R,S*)-[5-methyl-^{3}H]-AMPA (40-50 Ci/mmol; Perkin Elmer, Wellesley, MA) (iGluR1-4), [^{3}H]-SYM2081 (47.9 Ci/mmol, ARC, St.Louis, MO) (iGluR5) and [^{3}H]-kainic acid (58 Ci/mmol, Perkin Elmer) (iGluR6) as previously detailed.[10]

TEVC Electrophysiology. The rat AMPA-R clones iGluR1-4(*flip*) in the vector pGEMHE were used for preparation of high-expression cRNA transcripts for functional expression in oocytes as detailed previously.[11] Surgical procedures were conducted under the approval of the Danish Ministry of Justice Animal Experiments Inspectorate (2004/561-876). Mature female *Xenopus lævis* (Nasco, Fort Atkinson, WI) were anaesthetised using 0.1% ethyl 3-aminobenzoate methanesulfonate (tricaine) and ovaries were surgically removed. The ovarian tissue was dissected and treated with 2 mg/mL collagenase in nominally Ca^{2+}-free Barth's medium (in mM: 88 NaCl, 1 KCl, 0.33 $Ca(NO_3)_2$, 0.41 $CaCl_2$, 0.82 $MgSO_4$, 2.4 $NaHCO_3$, 10 HEPES, pH 7.4) for 2 h at room temperature. On the second day, oocytes were injected with 25-50 nL of (~ 1 mg/mL) cRNA and incubated in Barth's medium with 0.10 mg/mL gentamicin

(Sigma) and 1% penicillin-streptomycin (Life Technologies) at 17 ºC. Oocytes were typically used for recordings from 3–10 days post-injection and were voltage-clamped with the use of a two-electrode voltage clamp (TEVC) (GeneClamp 500B, Axon Instruments, Union City, CA) with both microelectrodes filled with 3 M KCl. Recordings were made while the oocytes were continuously superfused with Ca^{2+}-free frog Ringer's solution (in mM: 115 NaCl, 2 KCl, 1.8 $BaCl_2$, 5 HEPES, pH 7.0). Drugs were dissolved in Ca^{2+}-free frog Ringer's solution and added by bath application. Recordings were made at room temperature. Efficacy measurements were made at iGluR2(Q)$_i$ using saturating concentrations of agonist in the presence of 100 μM cyclothiazide in order to block receptor desensitisation (cyclothiazide EC_{50}: iGluR2(Q)$_i$ = 7.6 μM).[12] As a control for current rundown, oocytes were stimulated with 1 mM *L*-glutamate plus 100 μM cyclothiazide immediately prior to application of the test compound with a washout period of 5 - 10 min between applications. The maximum response of the test compound was then expressed as a fraction of the *L*-Glu stimulation. Concentration-response data for agonists were fit to a logistic equation to determine EC_{50} and Hill coefficient (n_H):

$$I = I_{max}/(1 + 10^{(\log[EC_{50}])}/10^{(\log [Agonist])})^{n_H}$$

where I is the measured current and I_{max} is the maximal steady-state current.

Mammalian Cell Culture. The CHO-K1 cell line was used for transfection of pCDNA3 derived expression vectors containing human iGluR5(Q)$_{1b}$ or iGluR1$_i$. The cells were cultured and maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) fetal calf serum and 2 mM *L*-proline in polystyrene culture flasks (12.5 or 25 cm^2) and in a humidified atmosphere of 5% CO_2/95% air, at 37 °C. The cells were transiently transfected using the LipofectAMINE PLUS™ (Life Technologies) transfection kit as described by the manufacturer. Cells were incubated over night and used the day after transfection. The cells were seeded onto glass cover slips (3.5 mm) pre-coated with poly-*D*-lysine which were placed

in Petri dishes and 2.5 mL of cell suspension (0.1×10^6 cells/mL) added. Cells were allowed to attach to the glass cover slips for 1 h before use.

Patch-Clamp Electrophysiology. Electrophysiological measurements were performed in voltage-clamp mode using conventional whole-cell patch-clamp techniques.[13]All data were obtained with an EPC-9 amplifier (HEKA-electronics, Lambrect, Germany) run by a Macintosh G3 computer. Experimental conditions and data acquisition were set and obtained using the PULSE-software accompanying the amplifier. Data was low-pass filtered and sampled directly to the hard disk. Pipettes were pulled from borosilicate glass using a horizontal electrode puller (Zeitz Instrumente, Augsburg, Germany), and the final pipette resistance was approximately 2 MΩ when filled with internal solution (in mM: 120 KCl, 31 KOH, 10 EGTA, 1.8 $MgCl_2$, 10 HEPES, pH 7.2) and submerged in the external solution (in mM: 140 NaCl, 5 KCl, 10 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, pH 7.4) used in the experiments.

Cover slips with cultured cells were transferred to a perfusion chamber mounted on the stage of an inverted microscope, and cells were continuously superfused with external solution at a rate of 2.5 mL/min. Compounds were dissolved in external solution and applied to the patched cell through double-barreled application pipettes fabricated from theta glass tubes (1.5 mm outer diameter, World Precision Instruments, Sarasota, FL) and mounted on a piezoelectric device (PZS-100HS, Burleigh Instruments, Quebec, Canada), which was controlled by the data-acquisition software. Complete solution exchange, evaluated by liquid junction potential changes, occurred in the order of 0.5 ms. One minute after the onset of the gravity flow a PULSE protocol was initiated and the current was recorded 3 times separated by 30 s waiting periods. The recording period was 150 ms during which the application pipette was switched to the test solution for 100 ms. After giga-seal formation (1-2 GΩ) and establishment of the whole-cell configuration, cells were held at a holding potential of -60 mV.

Antagonist Effects. For each cell, a control response induced by 3 mM *L*-Glu (iGluR5(Q)$_{1b}$) or 1 mM *L*-Glu (GluR1$_i$) was recorded followed by recordings of the glutamate-induced

responses in the presence of increasing concentrations of **7** or **5**. Because of the reversibility of the effect of **7** and **5** and the low, constant series resistance (< 5 MΩ), several concentrations could be tested on each cell.

Data Analyses. TEVC concentration-response data and radioligand binding data were analyzed using Grafit v3.00 (Erithacus Software Ltd., Horley, UK). Using the software GraphPad Prism v4.0 (GraphPad Software, San Diego, CA), agonist concentration-response data from patch clamp experiments were fit to the equation $I = I_{max}/(1+(EC_{50}/C)^{n})$, where I_{rmax} is the maximal response, EC_{50} is the compound concentration, C, producing a half-maximal response and *n* is the Hill coefficient. Inactivation concentration response data were fit to the equation $I = I_{max}/(1+(C/IC_{50})^{n})$, where I_{max} is the maximal response, IC_{50} denotes the compound concentration, C, yielding 50% inhibition and *n* is the Hill coefficient. SigmaStat v3.1 (SPSS Science, Chicago, IL) was employed for all statistical analyses. Values were considered statistically significantly different if $P< 0.05$.

Elemental Analyses

Compd	Formula	Calcd			Found		
		C	**H**	**N**	**C**	**H**	**N**
3	$C_{11}H_{15}N_3O_4$	52.17	5.97	16.59	52.05	6.26	16.54
4	$C_{15}H_{22}N_4O_6 \cdot ½\ H_2O$	49.58	6.38	15.42	49.76	6.23	15.57
5	$C_9H_{11}N_3O_4S$	42.02	4.31	16.33	41.91	4.55	16.20
6	$C_{12}H_{17}N_3O_4$	53.92	6.41	15.72	54.09	6.21	15.86
7	$C_9H_9N_3O_4S$	42.35	3.55	16.46	42.52	3.26	16.58
8	$C_{11}H_{11}N_3O_4 \cdot ⅓\ H_2O$	51.76	4.61	16.46	51.63	4.89	16.54
9	$C_{18}H_{17}N_3O_4$	63.71	5.05	12.38	63.60	5.38	12.46
10	$C_{16}H_{15}N_3O_5$	58.36	4.59	12.76	58.51	4.77	12.89
11	$C_9H_9N_3O_5$	45.19	3.79	17.57	45.32	4.06	17.41

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