Bioisosteric Modifications of 2-Arylureidobenzoic Acids: Selective Noncompetitive Antagonists for the Homomeric Kainate Receptor Subtype GluR5

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2-Arylureidobenzoic acids (AUBAs) have recently been presented as the first series of selective noncompetitive GluR5 antagonists. In this paper we have modified the acidic moiety of the AUBAs by introducing different acidic and neutral groups, and similarly, we have replaced the urea linker of the AUBAs with other structurally related linkers. Replacing the acid with neutral substituents led to inactive compounds in all instances, showing that an acidic moiety is necessary for activity. Replacing the carboxylic moiety in **2a** with a sulfonic acid (**5c**) or a tetrazole ring (**5d**) improved the potency at GluR5 receptors (compounds **5c** and **5d** showed IC_{50} values of 1.5 and 2.0 μM, respectively, compared to compound **2a** with IC_{50} = 4.8 μM). Compound **5c** did not show improved in vivo activity in the ATPA rigidity test compared to **2a**, whereas compound **5d** was 4 times more potent than **2a**. All compounds wherein the urea linker had been replaced showed lower or no activity. The results described extend the knowledge of structure−activity relationships for the AUBAs, and compound **5d** may prove to be a good candidate for studying GluR5 receptors in vitro and in vivo.

Introduction

(*S*)-Glutamic acid (Glu) is the main excitatory neurotransmitter in the central nervous system (CNS). The effects of Glu and other excitatory amino acids (EAAs) are mediated by three heterogeneous classes of ionotropic receptors (iGluRs) (*N*-methyl-D-aspartic acid (NMDA), 2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)-propionic acid (AMPA), and kainic acid (KA) receptors) and by three heterogeneous classes of metabotropic receptors (mGluRs) (namely, groups I, II, and III).[1] The iGluRs are composed of different subunits that assemble into tetrameric structures that form functional cation channels. Seven NMDA subunits (NR1, NR2A−D, and NR3A, NR3B), four AMPA subunits (GluR1−4), and five KA subunits (GluR5−7, KA1, and KA2) have been cloned and characterized.[2]

All three classes of iGluRs have been implicated in different diseases, and although NMDA and AMPA selective ligands have held much of the research focus, KA selective ligands and in particular ligands for the GluR5 subunit have received increasing attention following the cloning and ongoing physiological characterization of GluR5 and other KA receptors.[1,3,4] GluR5 selective antagonists have been proposed to be potentially useful in the treatment of various diseases such as ischemic conditions,[5,6] pain,[7] epilepsy,[8] and migraine.[9] A particular advantage of GluR5 selective antagonists is that blocking this subunit has so far not been associated with serious side effects, whereas non-subunit selective AMPA and NMDA antagonists have been found to induce, for example, ataxia and psychotomimetic effects, respectively.[1]

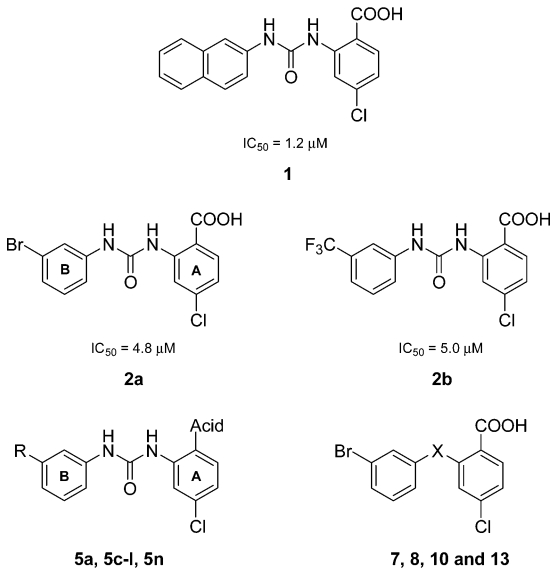

Figure 1. Structures of the noncompetitive GluR5 selective antagonists **1**, **2a**, and **2b** and generalized structures of the modified AUBAs presented in this paper. See Tables 1 and 2 for lists of substituents.

We have previously published a series of 2-arylureidobenzoic acids (AUBAs) that showed GluR5 antagonistic activity in vitro and in vivo.[10] Therein, we investigated the structure−activity relationship (SAR) around the two aromatic rings in the AUBAs and

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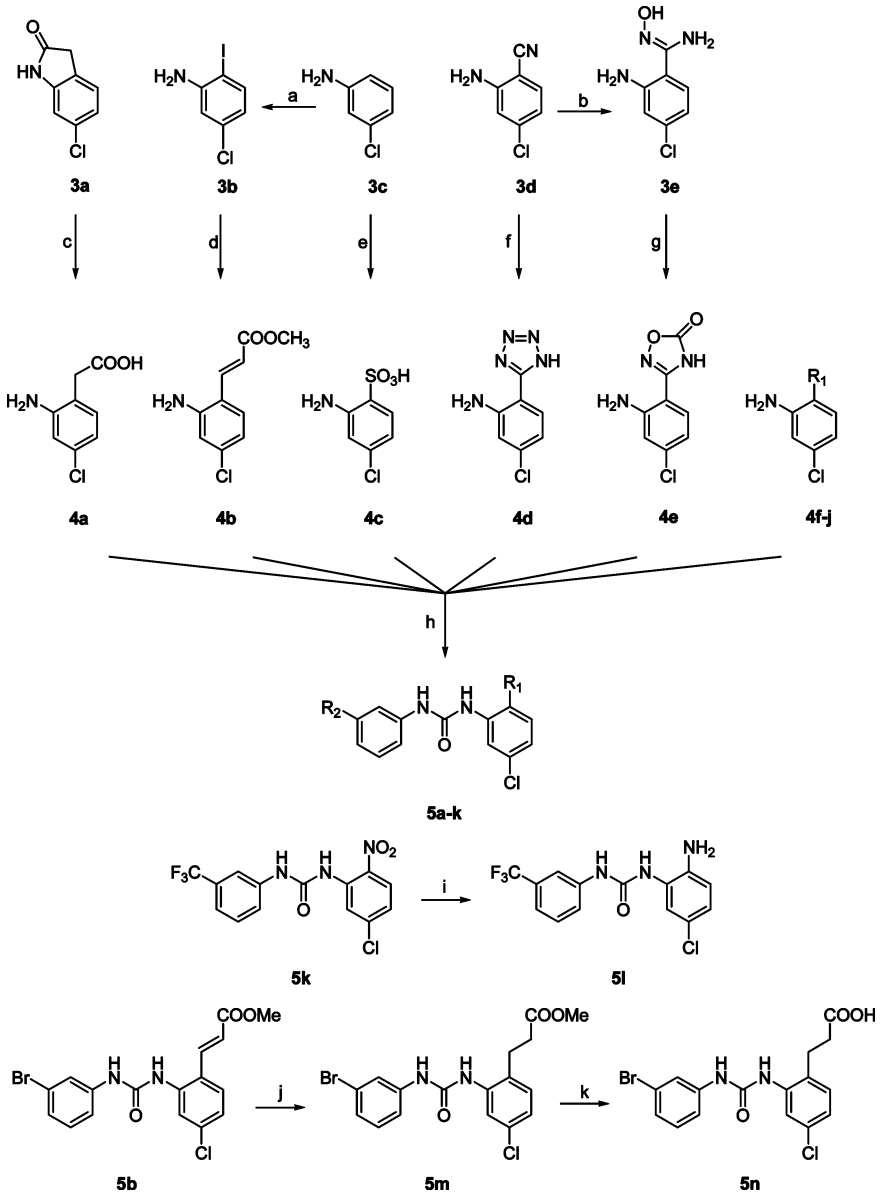
Scheme 1[a]



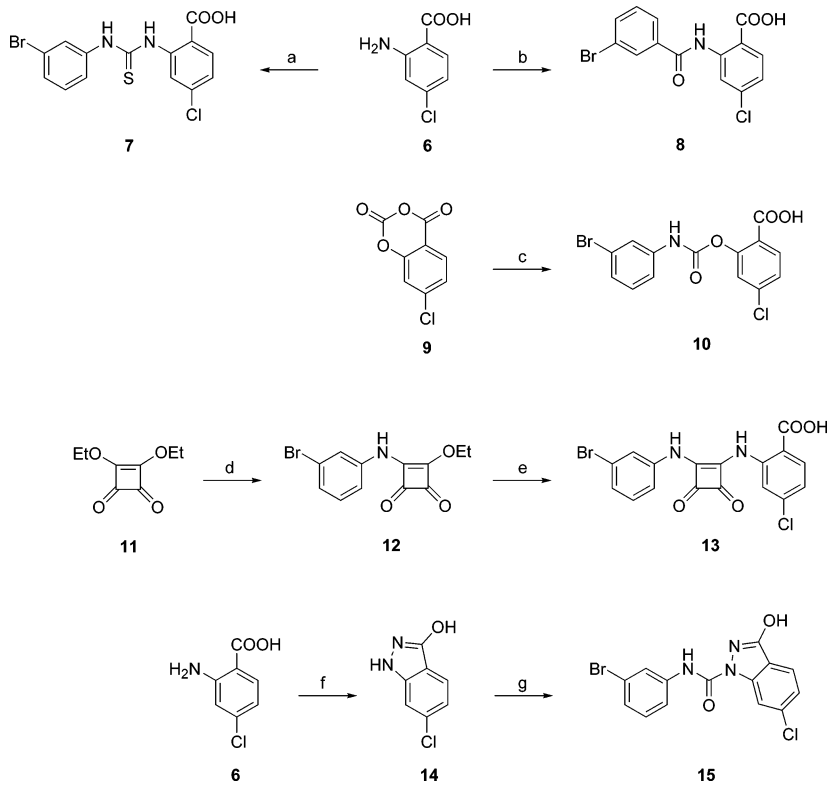
[a] Reagents and conditions: (a) NIS, AcOH, room temp; (b) $NH_2OH{\cdot}HCl$, $NaHCO_3$, EtOH, reflux; (c) NaOH, H_2O, reflux; (d) methyl acrylate, Pd/C, K_2CO_3, DME, 100 °C; (e) H_2SO_4 (conc), heat; (f) NaN_3, $Et_3N{\cdot}HCl$, toluene; (g) NaOEt, $(EtO)_2CO$, EtOH, reflux; (h) 3-bromophenyl isocyanate or 3-trifluoromethylphenyl isocyanate, THF, room temp; (i) H_2, Raney Ni, EtOH; (j) H_2, Pd/C, EtOH; (k) NaOH, EtOH, room temp. See Table 1 for a list of substituents.

optimized these for in vitro GluR5 potency. However, the compound that was most potent in vitro (**1**, see Figure 1) turned out to be inactive in vivo (ED_{50} > 30 mg/kg in the ATPA-induced rigidity test), whereas compound **2a**, which was 5 times less potent in vitro compared to **1**, was active in vivo (ED_{50} = 4 and 24 mg/kg after 5 and 30 min, respectively). This exemplifies the difficulties in correlating in vitro with in vivo activities and calls for further optimization and attention to the pharmacokinetic properties of the compounds. Here, we present a new series of AUBA derivatives where the acid moiety on ring A (see Figure 1) has been replaced by other acidic and neutral groups, thus exploring the pharmacological effects of using moieties other than benzoic acid, which is prone to conjugation and other metabolic processes. Compounds **5a, 5c**−**l**, and **5n** are analogues of the two equipotent analogues from the first series of AUBAs, **2a** or **2b**. Furthermore, we have exchanged the urea moiety, which links the two aromatic ring systems of the AUBAs, for other linkers of similar size and structure to yield compounds **7**, **8**, **10**, **13**, and **15**. These modifications of the acid and urea moieties, combined with the observations around the two aryl rings from our previous paper,[10] constitute a more comprehensive SAR for the AUBAs as GluR5 antagonists.

Results

Chemistry. The compounds with modified acid moieties (**5a**−**k**) were prepared by condensing the appropriately substituted anilines (**4a**−**j**) with 3-bromophenyl isocyanate or 3-trifluoromethylphenyl isocyanate (see Scheme 1). Attempts to hydrogenate **5k** using Pd/C as catalyst gave a complex mixture of compounds, but exchanging Pd/C with Raney Ni yielded 1-(2-amino-5-

Scheme 2[a]



[a] Reagents and conditions: (a) 3-bromophenyl isothiocyanate, Et_3N, THF; (b) 3-bromobenzoyl chloride, pyridine, room temp; (c) 3-bromoaniline, THF, room temp; (d) 3-bromoaniline, EtOH, room temp; (e) 2-amino-4-chlorobenzoic acid, Et_3N, MeCN, reflux; (f) (1) $NaNO_2$, HCl, H_2O, 0 °C; (2) $NaSO_3$, H_2O, room temp; (3) HCl, room temp; (g) 3-bromophenyl isocyanate, pyridine, room temp.

chlorophenyl)-3-(3-trifluoromethylphenyl)urea (**5l**) as the only product in a good yield. Phenylpropionic acid **5n** was prepared from the acrylic acid ester **5b** in two steps. Catalytic hydrogenation of **5b** using Pd/C yielded **5m**, which was then hydrolyzed with NaOH to yield **5n**. The anilines **4f**−**j** were commercially available, and anilines **4a**−**e** were prepared as follows. (2-Amino-4-chlorophenyl)acetic acid (**4a**) was prepared from 6-chlorooxindole (**3a**) by reflux in aqueous NaOH and precipitation of **4a** as a zwitterion. 3-(2-Amino-4-chlorophenyl)acrylic acid methyl ester (**4b**) was prepared in two steps. 5-Chloroaniline (**3c**) was treated with *N*-iodosuccinimide (NIS) to yield 5-chloro-2-iodoaniline (**3b**), which was subsequently cross-coupled with acrylic acid methyl ester to yield **4b**, using Pd/C as a catalyst.[11] Attempts to couple **3b** with propynoic acid methyl ester under modified[12] Sonogashira conditions failed. 2-Amino-4-chlorobenzenesulfonic acid (**4c**) was prepared from 3-bromoaniline (**3c**) by modification of the sulfonation procedure of Allen et al.[13] Tetrazole-substituted aniline **4d** was prepared from 2-amino-4-chlorobenzonitrile (**3d**) as described by Koguro et al.[14] Oxadiazolone **4e** was prepared in two steps. Nucleophilic addition of hydroxylamine to 2-amino-4-chlorobenzonitrile (**3d**) gave 2-amino-4-chloro-*N*-hydroxybenzamidine (**3e**),[15] which was treated with diethyl carbonate under basic conditions to form the 4*H*-[1,2,4]-oxadiazol-5-one ring of **4e**.[16]

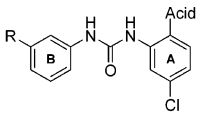
Thiourea **7** and amide **8** were prepared from aniline **6** by reaction with 3-bromophenyl isothiocyanate and 3-bromobenzoyl chloride, respectively (Scheme 2). Attempts to synthesize carbamate **10** from 4-chlorosalicylic acid and 3-bromophenyl isocyanate were unsuccessful because the reaction conditions that were necessary to make the salicylic acid react (1 equiv of Et_3N in THF at 50 °C) caused immediate and quantitative cyclization of the desired product into 3-(3-bromophenyl)-7-chlorobenzo[*e*][1,3]oxazine-2,4-dione. This is analogous to the results obtained previously when applying these conditions to similar compounds.[10] However, **10** could be prepared from 4-chlorosalicylic acid in two steps through 7-chlorobenzo[1,3]dioxine-2,4-dione (**9**), which reacts with 3-bromoaniline at the carbamate carbonyl as reported in the literature for similar compounds.[17]

Compound **13** was prepared by reacting 3,4-diethoxycyclobut-3-ene-1,2-dione (**11**) with 3-bromoaniline and aniline **6** successively. The ring-fused analogue **15** was synthesized by condensing 3-bromophenyl isocyanate with 6-chloro-1*H*-indazol-3-ol **14**, which was synthesized by reacting anthranilic acid **6** with $NaNO_2$.

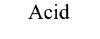
In Vitro Pharmacology. The antagonistic effects of the compounds on different recombinant homomeric rat AMPA (GluR1−4) and human KA receptors (GluR5−6), stably expressed in HEK293 cells, were evaluated by functional assays using the FLIPR (fluorescent imaging plate reader) technology. The antagonistic activity of the test compounds was quantified as the inhibition of the increase in intracellular Ca^{2+} concentration, measured by light emission from a Ca^{2+} sensitive fluorophore, following the addition of a known agonist (Glu for GluR1−4 and domoic acid for GluR5,6) to cells expressing the appropriate receptor.

All the compounds were screened for activity at GluR5 (see IC_{50} values in Tables 1 and 2). None of the neutral compounds **5f**−**l**, bearing CO_2Me, OH, CH_2OH, CN, NO_2, or NH_2 instead of the carboxylic acid group of **2a**

Table 1. Antagonist Effects of Compounds with Different Acidic or Neutral Substitutions for the Carboxylic Acid Group of Compounds **2a** and **2b**[a]



2a, 2b, 5a, 5c-l, 5n

Compd	Acid	R	IC_{50} (μM)
2a	—COOH	—Br	4.8 ± 1.1
2b	—COOH	—CF_3	5.0 ± 1.1
5a		—Br	3.6 ± 0.28
5n		—Br	> 30
5c	—SO_3H	—Br	1.5 ± 0.33
5d		—Br	2.0 ± 0.53
5e		—Br	4.6 ± 0.61
5f	—CO_2Me	—Br	> 30
5g	—OH	—CF_3	> 30
5h	—CH_2OH	—CF_3	> 10
5i	—CN	—CF_3	> 3
5j	—NO_2	—Br	> 3
5k	—NO_2	—CF_3	> 3
5l	—NH_2	—CF_3	> 30

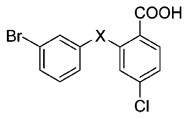
[a] IC_{50} values were determined with a FLIPR assay using HEK293 cells expressing GluR5. Results are the mean ± SEM for at least three separate experiments.

and **2b**, showed any activity at the concentrations tested. The concentrations tested of the neutral compounds were limited by their low aqueous solubility because the lack of an acidic moiety lowered the solubility markedly. All but one of the acidic compounds were active at the GluR5. The tetrazole **5d** was approximately twice as potent as **2a**, and the oxadiazolone **5e** was equipotent with **2a**. The phenylacetic acid **5a** was also equipotent with **2a**, but the phenylpropanoic acid analogue **5n** was inactive. The sulfonic acid analogue **5c** was about 3 times more potent than **2a**.

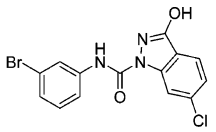
Of the compounds with modified urea moieties, only the thiourea **7** and amide **8** were active, although 2 and 4 times less potent than **2a**, respectively. The carbamate **10**, the dione analogue **13,** and the ring fused analogue **15** were all inactive.

The compounds that were equally or more potent than **2a** in the GluR5 assay (**5a**, **5c**−**e**) were further characterized in functional assays (FLIPR) using GluR1−4 and GluR6 and in binding studies using recombinant receptors and the GluR5-selective agonist [^{3}H]-(*RS*)-2-amino-3-(3-hydroxy-5-*tert*-butyl-4-isoxazolyl)propionic acid ([^{3}H]ATPA) (see Table 3). None of the compounds showed any GluR1−4 antagonistic activity at 30 μM and only weak antagonism (less than 50%) at 100 μM. The potency at GluR6 could only be determined for **5c**, for which the GluR6 potency was about 25 times lower than at the GluR5. The other compounds were not sufficiently soluble to enable accurate determination of their IC_{50} for GluR6 (see Table 3). No significant inhibition of [^{3}H]ATPA binding to GluR5 receptors was observed at the concentrations tested (3−30 μM).

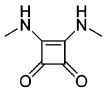
Table 2. GluR5 Antagonist Effects, Measured by FLIPR Assay on HEK293 Cells, of Compounds with Different Linker Moieties[a]



7,8,10 and 13



15

Compd	X	IC_{50} (μM)
7		12 ± 1.0
8		19 ± 2.1
10		> 30
13		> 30
15		> 50

[a] IC_{50} values were determined with a FLIPR assay using HEK293 cells expressing GluR5. Results are the mean ± SEM for at least three separate experiments.

In Vivo Pharmacology. The in vivo activities of compounds **5a** and **5c**−**e** were evaluated in the ATPA-induced rigidity test in mice. The test compounds were administered iv 30 min prior to the administration of the GluR5 agonist ATPA, and the degree of rigidity induced by ATPA was observed. The benzoic acid **2a** has previously been reported to have an ED_{50} of 24 mg/kg in this model.[10] The sulfonic acid **5c** had a potency similar to **2a** (ED_{50} = 29 mg/kg), whereas the tetrazole **5d** was notably more potent, having an ED_{50} of 5.5 mg/kg. Phenylacetic acid **5a** and oxadiazolone **5e** were inactive at the doses tested.

Discussion

Acid and urea modified AUBA analogues were designed and synthesized in a concise manner. A series of 12 compounds (**5a**, **5c**−**l**, and **5n**), wherein the acid moiety of **2a** or **2b** is replaced by other acidic or neutral groups, have been synthesized. Care was taken to introduce diverse acidic functionalities, e.g., homologues

Table 3. Potencies of Selected Compounds in Functional Assays for GluR1−6 (FLIPR Assays and Electrophysiology on GluR5),[a] [^{3}H]ATPA Binding (All as IC_{50} in μM),[a] and ATPA Rigidity (as ED_{50} in mg/kg)

		Antagonism in FLIPR assay			[^{3}H]ATPA Binding	ATPA Rigidity
Compd	Acid	GluR1–4	GluR5	GluR6	GluR5	30 min
1		> 100	1.2 ± 0.35	62 ± 10	>30	>30
2a	—COOH	> 100	4.8 ± 1.1	28 ± 6.9	>30	24
5a		> 100	3.6 ± 0.28	> 50	>30	>30
5c	—SO_3H	> 100	1.5 ± 0.33	37 ± 7.8	>30	29
5d		> 100	2.0 ± 0.53	> 10	>30	5.5
5e		> 100	4.6 ± 0.61	> 30	>30	> 100

[a] Results are the mean ± SEM for at least three separate experiments.

(**5a** and **5n)**, heterocycles (**5d** and **5e**), and a sulfonic acid (**5c**). Furthermore, mesoionic (**5j** and **5k**) and other neutral compounds (**5f**−**i** and **5l**) were prepared. An additional five compounds where the urea moiety of **2a** was modified were synthesized.

As demonstrated by the activities of compounds **2a** and **2b** (Table 1), it is not crucial for GluR5 activity whether ring B of the AUBAs bears a 3-bromo or 3-trifluoromethyl substitutent (IC_{50} values of 4.8 and 5.0, respectively). We have included seven compounds bearing neutral moieties instead of the carboxylic acid of **2a** and **2b**, and it can be concluded that an acidic functionality is needed for GluR5 activity (Table 1).

All the acidic analogues, except the two-carbon homologue **5n**, showed GluR5 activity comparable to that of **2a**. The one-carbon homologue **5a** was about equipotent with **2a** (3.6 versus 4.8 μM, respectively) and more selective than **2a** for GluR5 over GluR6. The two-carbon homologue **5n** was found to be inactive at the concentration tested (30 μM), suggesting some specific limitations with regard to the binding site of the acidic moiety. The sulfonic acid **5c** was approximately 3 times more potent than **2a**, further establishing that ionic interactions are needed for GluR5 activity. The oxadiazolone **5e** was about equipotent with **2a,** but the tetrazole **5d** was more than twice as potent as **2a**. The higher affinity of the tetrazole might be attributed to the increased lipophilicity of the tetrazolyl anion compared to the carboxylate anion because of the delocalization opportunities in the former and because of the possibilities of making multiple hydrogen bonds to the receptor through the ring nitrogens.[18]

Thus, the receptor can accommodate acidic groups that are considerably larger than the carboxylate. Because the presence of ionized groups enhances the solvation of ligands in the aqueous environment outside the receptor (makes the free energy of solvation more negative) and thereby inherently decreases the affinity of the compounds, we investigated nonionized alternatives to replace the carboxylate. The mesoionic nitro group was our prime candidate in this regard because it is a hydrogen bond acceptor but does not have a net charge. However, the nitro compounds **5j** and **5k** were not active at the concentrations tested (3 μM). It also became apparent that the presence of acidic functionalities was necessary for adequate aqueous solubility; compounds **5f**−**l** were only soluble in low concentrations in the assay buffers.

Most of the compounds in which the urea moiety was modified were inactive. However, limited activity was retained for thiourea **7** and amide **8**, which were about 2 and 3 times less potent than **2a**, respectively. These results indicate that although urea is the best linker tested, it is possible to modify the linker without causing complete loss of activity. The ring-fused analogue **15** was designed on the basis of the observation that an intramolecular hydrogen bond between the proximal −NH of the urea and the carboxylate anion seems very favorable, and therefore, the carboxylic acid and the nitrogen can be envisioned to form a six-membered ring. Compound **15** forms an analogous five-membered 3-hydroxypyrazole ring, which restricts the conformational freedom of the acidic functionality. Unfortunately **15** was completely inactive at GluR5, indicating that this particular restriction is not suitable.

The noncompetitive activity observed for the previous series of AUBAs[10] was confirmed for the present compounds by lack of inhibition of [^{3}H]ATPA binding to GluR5 (Table 3). The in vivo effects of the acidic bioisosteres **5a**,**c**−**e** were measured using ATPA-induced rigidity. Sulfonic acid **5c** was about equipotent with the carboxylic acid **2a**. However, the tetrazole **5d** gave 4-fold higher in vivo activity compared to **2a**. Part of this can be attributed to the higher in vitro activity of **5d** (2.0 versus 4.8 μM, respectively), but it should also be considered that tetrazoles are known to be more metabolically stable than carboxylic acids and therefore more likely to be active in vivo.[18] Furthermore, the more lipophilic tetrazole may improve penetration of the blood−brain barrier compared to the carboxylic acid group. Surprisingly, the phenylacetic acid **5a** and the oxadiazolone **5e** were devoid of in vivo activity (tested

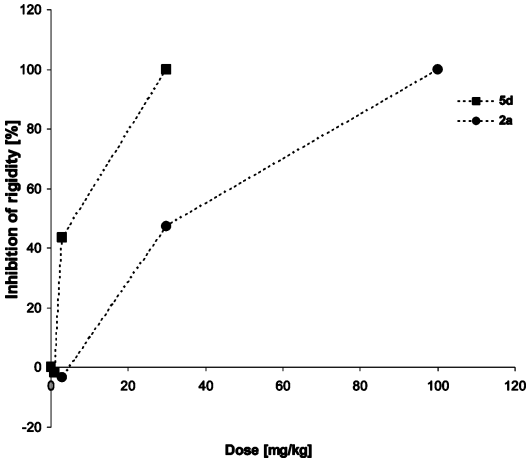


Figure 2. Comparison of the dose−response curves, obtained in the in vivo ATPA-induced rigidity test, for carboxylic acid **2a** (ED_{50} = 24 mg/kg) and tetrazole **5d** (ED_{50} = 5.5 mg/kg).

up to 30 and 100 mg/kg, respectively), although they were equipotent with **2a** in vitro. This was especially disappointing in the case of the oxadiazolone **5e** because this heterocycle has previously been used as a lipophilic carboxylic bioisostere with good results, for example, in ACE inhibitors.[19]

Conclusion

The acid and urea moieties of AUBAs, which are GluR5 selective antagonists, have been modified in order to elucidate the SAR of these compounds and as an attempt to improve their in vitro activity. The importance of the acidic moiety has been established, as all the neutral compounds were inactive, and the structural requirements of the receptor with respect to the acidic group do not seem to be very stringent, as all but one of the acidic compounds retained or were increased in activity compared to the carboxylic acid **2a**. Similarly, the urea linker can be modified without abolishing all activity, but the urea moiety still yields the most active compounds. When considered together with our previous publication,[10] SAR information has been gathered for all the components of the AUBAs, that is, both aromatic rings A and B, the urea linker, and the acid moiety.

The tetrazolyl group (analogue **5d**) improved the in vivo potency significantly compared to the previous carboxylic acid derivatives (Table 1 and Figure 2). This makes **5d** a more suitable candidate for in vivo characterization of GluR5 pharmacology than **2a**, **2b**, or **1**.

Experimental Section

Chemistry. All reagents are commercially available unless stated otherwise. All reactions were carried out under nitrogen atmosphere. Melting points were measured on a capillary melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Varian 300 MHz spectrometer. Residual solvent peaks were used as an internal reference in the NMR spectra. Elemental analyses were within ±0.4% of the theoretical value unless stated otherwise.

General Procedure A: Synthesis of AUBAs 5a−k. The appropriate isocyanate (2.1 mmol) and the appropriate aniline (2.0 mmol) were dissolved/suspended in dry THF (10 mL), toluene (100 mL), or DMSO (10 mL), and the mixture was stirred at room temperature until one or both starting materials could not be detected by TLC (typically 2−3 h when run in THF and DMSO but 24 h when run in toluene). If both starting materials were detected after 24 h, then the reaction mixture was refluxed until the reaction was complete. The solution was cooled, and the product precipitated with heptane when necessary. The product was then filtered off, washed, and recrystallized as described below for each compound.

5-Chloro-2-iodoaniline (3b). 3-Chloroaniline (**3c**) (30.0 g, 236 mmol) and *N*-iodosuccinimide (NIS) (55.8 g, 248 mmol) were dissolved in glacial acetic acid (250 mL) and stirred for 12 h. The mixture was evaporated down to 50 mL, and succinic anhydride was precipitated from the solution by addition of EtOAc and heptane and filtered off. The mother liquor was washed with 1 M NaOH, dried with Na_2SO_4, and evaporated. The product was recrystallized from Et_2O/heptane to yield faintly yellow crystalline **3b** (42 g, 70%). Mp: 64−66 °C (lit. 40−42 and 75−77 °C).[20,21] The ^{1}H NMR spectrum was in agreement with the literature.[21] Anal. (C_6H_5ClIN) C, H, N.

2-Amino-4-chloro-*N*-hydroxybenzamidine (3e). 2-Amino-4-chlorobenzonitrile (**3d**) (5.3 g, 35 mmol) was dissolved in EtOH (180 mL) by heating. An aqueous solution of hydroxylamine hydrochloride (4.9 g, 70 mmol) and sodium bicarbonate (11.1 g, 105 mmol) was added, and the mixture was refluxed for 8 h. The solvent was evaporated and the product was crystallized by suspension in water (100 mL) to yield colorless crystalline **3e** (5.6 g, 86%). Mp: 131−133 °C (lit. 127−128 °C).[22] ^{1}H NMR (DMSO-d_6) δ 9.66 (1H, s), 7.36 (1H, d, J = 8.5 Hz), 6.70 (1H, s), 6.55 (1H, br s), 6.52 (1H, d, J = 8.5 Hz), 5.78 (2H, br s). Anal. ($C_7H_8ClN_3O$) C, H, N.

3-(2-Amino-4-chlorophenyl)acrylic Acid Methyl Ester (4b). Iodoaniline **3b** (1.25 g, 5.0 mmol), methyl acrylate (750 mg, 7.50 mmol), K_2CO_3 (1.05 g, 7.50 mmol), and Pd/C (630 mg, 0.30 mmol) were dissolved in DME (20 mL) and stirred at 100 °C for 48 h. The mixture was diluted with EtOAc and filtered through Celite. The solvent was evaporated and the product was recrystallized from EtOAc/heptane to yield colorless crystalline **4b** (0.95 g, 90%). Mp: 115−121 °C (lit. 124−125 °C).[23] The ^{1}H NMR spectrum was in agreement with the literature.[23] Anal. ($C_{10}H_{10}ClNO_2$) C, H. N: calcd, 6.62; found, 6.01.

2-Amino-4-chlorobenzenesulfonic Acid (4c). Concentrated sulfuric acid (specific gravity 1.84) (13.9 mL, 250 mmol) was diluted with 100 mL of water, and 3-chloro-aniline (**3c**) (31.9 g, 250 mmol) was added over 30 min while the temperature was kept at 85 °C. During the addition some precipitation occurred. The suspension was allowed to cool to room temperature, whereupon heavy precipitation occurred. The suspension was filtered to yield a colorless crystalline solid that was heated slowly to 200 °C under vacuum (water aspirator) and heated for 7 h. Water was lost from the crystalline material. The solid was allowed to cool and then dissolved in dilute NaOH. The product was precipitated by addition of 4 M HCl, filtered off, and dried to yield colorless crystalline **4c** (25 g, 48%). Mp: >250 °C (lit. >310 °C).[24] ^{1}H NMR (DMSO-d_6) δ 7.48 (1H, d, J = 8.5 Hz), 6.83 (1H, s), 6.71 (1H, d, J = 8.5 Hz). Anal. ($C_6H_6ClNO_3S$) C, H, N.

3-(2-Amino-4-chlorophenyl)-4*H*-[1,2,4]oxadiazol-5-one (4e). Sodium metal (0.92 g, 40 mmol) was dissolved in 70 mL of EtOH, and **3e** (3.71 g, 20 mmol) was added, followed by diethyl carbonate (9.44 g, 80 mmol). The mixture was refluxed for 18 h and then reduced to dryness under vacuum. The residue was triturated with 0.5 M HCl, and the product was filtered off. The product was then triturated with toluene and filtered off to yield red-brown crystalline **4a** (3.4 g, 80%). Mp: 159−161 °C. ^{1}H NMR (DMSO-d_6) δ 7.52 (1H, d, J = 8.5 Hz), 6.94 (1H, s), 6.68 (1H, d, J = 8.5 Hz). Anal. ($C_7H_8ClN_3O\cdot H_2O$) C, H, N.

{**2-[3-(3-Bromophenyl)ureido]-4-chlorophenyl**}**acetic Acid (5a).** 6-Chlorooxindole (**3a**) (2.50 g, 14.9 mmol) was suspended in 4 M aqueous NaOH (30 mL), the mixture was refluxed for 6 h and allowed to cool, and the product was filtered off as the sodium salt. The solid was dissolved in water (100 mL) and precipitated as an HCl salt by addition of 4 M HCl at 0 °C. Crude **4a** was filtered off and dried under vacuum to yield colorless crystals (2.0 g). Mp: 251−254 °C (dec). ^{1}H

NMR (D_2O) δ 10.00 (1H, s), 8.89 (1H, s), 8.36 (1H, d, J = 8.5 Hz), 8.29 (1H, t, J = 8.0 Hz), 8.21 (1H, d, J = 8.0 Hz), 3.55 (2H, s). Crude **4a** (700 mg, 3.8 mmol) was dissolved in pyridine (25 mL), 1-bromophenyl 3-isocyanate (811 mg, 4.1 mmol) was added dropwise over 10 min, and the mixture was stirred for 90 min. The mixture was diluted with 150 mL of EtOAc and washed twice with 1 M HCl. The volume of the organic phase was reduced under vacuum and the product was recrystallized from EtOAc/heptane to yield colorless crystalline **5a** (680 mg, 47%). Mp: 195−196 °C. ^{1}H NMR (DMSO-d_6) δ 12.57 (1H, s), 9.41 (1H, s), 8.20 (1H, s), 7.93 (1H, d, J = 2 Hz), 7.89 (1H, t, J = 2 Hz), 7.26 (1H, m), 7.16 (1H, dt, J = 7.5 Hz, 2 Hz), 7.08 (1H, dd, J = 8.5 Hz, 2 Hz), 3.64 (2H, s). Anal. ($C_{15}H_{12}BrClN_2O_3 \cdot {}^1/_3H_2O$) C, H, N.

3-{2-[3-(3-Bromophenyl)ureido]-4-chlorophenyl}-acrylic Acid Methyl Ester (5b). 5b was prepared according to general procedure A to yield a colorless crystalline solid. Yield: 93%. Mp: 186−190 °C. ^{1}H NMR (DMSO-d_6) δ 9.23 (1H, s), 9.06 (1H, s), 7.84 (4H, m), 7.33 (2H, m), 7.23 (1H, t, J = 8.0 Hz), 7.15 (1H, d, J = 8.0 Hz), 6.58 (1H, d, J = 15.5 Hz), 3.72 (3H, s). Anal. ($C_{17}H_{14}BrClN_2O_3$) C, H, N. C: calcd, 49.84; found, 50.35. N: calcd, 6.84; found, 6.27.

2-[3-(3-Bromophenyl)ureido]-4-chlorobenzenesulfonic Acid, Triethylammonium Salt (5c). 5c was prepared according to a modified version of general procedure A (1 equiv of Et_3N was added to the reaction mixture along with the starting materials) to yield a colorless crystalline solid. Yield: 70%. Mp: 178−179 °C. ^{1}H NMR (DMSO-d_6) δ 10.00 (1H, s), 9.49 (1H, s), 8.85 (1H, br s), 8.13 (1H, s), 7.91 (1H, s), 7.66 (1H, d, J = 8.5 Hz), 7.47 (1H, d, J = 8.0 Hz), 7.23 (1H, t, J = 8.0 Hz), 7.14 (1H, d, J = 8.0 Hz), 7.02 (1H, d, J = 8.5 Hz), 3.09 (6H, q, J = 7.5 Hz), 1.16 (9H, t, J = 7.5 Hz). Anal. ($C_{13}H_{10}BrClN_2O_4S \cdot C_6H_{15}N$) C, H, N.

1-(3-Bromophenyl)-3-[5-chloro-2-(1*H*-tetrazol-5-yl-phenyl]urea (5d). 5d was prepared from 5-chloro-2-(1*H*-tetrazol-5-yl)phenylamine[14] (**4d**) and 3-bromophenyl isocyanate by general procedure A to yield a colorless crystalline solid. Yield: 78%. Mp: 197−199 °C. ^{1}H NMR (DMSO-d_6) δ 10.08 (1H, s), 9.97 (1H, s), 8.42 (1H, s), 7.91 (2H, m), 7.42 (1H, d, J = 8.0 Hz), 7.33 (1H, d, J = 8.0 Hz), 7.26 (1H, t, J = 8.0 Hz), 7.19 (1H, d, J = 8.0 Hz). Anal. ($C_{14}H_{10}BrClN_6O$) C, H, N.

1-[5-Chloro-2-(5-oxo-4,5-dihydro-[1,2,4]oxadiazol-3-yl)-phenyl]-3-(3-bromophenyl)urea (5e). 5e was prepared from **4e** according to general procedure A to yield a colorless crystalline solid. Yield: 63%. Mp: 246−248 °C (decp). ^{1}H NMR (DMSO-d_6) δ 13.07 (1H, s), 9.97 (1H, s), 9.08 (1H, s), 8.32 (1H, s), 7.67 (1H, d, J = 8.5 Hz), 7.37 (1H, d, J = 8.0 Hz), 7.32 (1H, d, J = 8.5 Hz), 7.27 (1H, t, J = 8.0 Hz), 7.20 (1H, d, J = 8.0 Hz). Anal. ($C_{15}H_{10}BrClN_4O_3 \cdot 1{}^1/_4H_2O$) C, H, N.

2-[3-(3-Bromophenyl)ureido]-4-chlorobenzoic Acid Methyl Ester (5f). 5f was prepared according to general procedure A to yield a colorless crystalline solid. Yield: 88%. Mp: 164−166 °C. ^{1}H NMR (DMSO-d_6) δ 10.22 (1H, s), 10.17 (1H, s), 8.50 (1H, s), 7.92 (1H, d, J = 8.5 Hz), 7.88 (1H, s), 7.40 (1H, d, J = 8.5 Hz), 7.25 (1H, t, J = 8.0 Hz), 7.17 (1H, d, J = 8.0 Hz), 7.11 (1H, d, J = 8.5 Hz), 3.88 (3H, s). Anal. ($C_{15}H_{12}BrClN_2O_3$) C, H, N.

1-(5-Chloro-2-hydroxyphenyl)-3-(3-trifluoromethylphenyl)urea (5g). 5g was prepared according to general procedure A to yield a colorless crystalline solid. Yield: 96%. Mp: 177−178 °C. ^{1}H NMR (DMSO-d_6) δ 10.36 (1H, s), 9.75 (1H, s), 8.39 (1H, s), 8.16 (1H, s), 8.03 (1H, s), 7.51 (2H, s), 7.32 (1H, s), 6.85 (2H, s). Anal. ($C_{14}H_{10}ClF_3N_2O_2$) C, H, N.

1-(5-Chloro-2-hydroxymethylphenyl)-3-(3-trifluoromethylphenyl)urea (5h). 5h was prepared according to general procedure A to yield a colorless crystalline solid. Yield: 74%. Mp: 217−218 °C. ^{1}H NMR (DMSO-d_6) δ 9.71 (1H, s), 8.30 (1H, s), 8.01 (1H, s), 7.88 (1H, d, J = 8.5 Hz), 7.59 (1H, d, J = 8.5 Hz), 7.51 (1H, t, J = 8.5 Hz), 7.38 (1H, s), 7.28 (2H, m), 4.52 (2H, s). Anal. ($C_{15}H_{12}ClF_3N_2O_2$) C, H, N.

1-(3-Bromophenyl)-3-(5-chloro-2-cyanophenyl)urea (5i). 2-Amino-4-chlorobenzonitrile (**4i**) (450 mg, 2.95 mmol), 3-bromophenyl isocyanate (594 mg, 3.00 mmol), and *p*-toluenesulfonic acid (52 mg, 0.30 mmol) were suspended in toluene, and the suspension was stirred at 100 °C for 1 h. When the mixture was cooled, the product precipitated and was filtered off, dried, and recrystallized (acetone/water) to yield yellow crystalline **5i** (795 mg, 76%). Mp: 221−222 °C. ^{1}H NMR (DMSO-d_6) δ 9.72 (1H, s), 8.97 (1H, s), 8.26 (1H, s), 7.89 (1H, s), 7.84 (1H, d, J = 8.5 Hz), 7.30 (3H, m), 7.24 (1H, t, J = 7.5 Hz). Anal. ($C_{14}H_9BrClN_3O$) C, H, N.

1-(3-Bromophenyl)-3-(5-chloro-2-nitrophenyl)urea (5j). 5-Chloro-2-nitroaniline (**4j**) (518 mg, 3.00 mmol), 3-bromophenyl isocyanate (693 mg, 3.00 mmol), and *p*-toluenesulfonic acid (52 mg, 0.30 mmol) were suspended in toluene, and the suspension was stirred at 100 °C for 1 h. When the mixture was cooled, the product precipitated and was filtered off, dried, and recrystallized (acetone/water) to yield yellow crystalline **5j** (830 mg, 79%). Mp: 199−200 °C. ^{1}H NMR (DMSO-d_6) δ 10.23 (1H, s), 9.81 (1H, s), 8.50 (1H, s), 8.18 (1H, d, J = 9.0 Hz), 7.88 (1H, s), 7.36 (1H, d, J = 8.0 Hz), 7.30 (2H, m), 7.24 (1H, d, J = 8.0 Hz). Anal. ($C_{13}H_9BrClN_3O_3$) C, H, N.

1-(5-Chloro-2-nitrophenyl)-3-(3-trifluoromethylphenyl)-urea (5k). 5-Chloro-2-nitroaniline (**4j**) (1036 mg, 6.00 mmol), 3-trifluoromethylphenyl isocyanate (1.12 g, 6.00 mmol), and *p*-toluenesulfonic acid (52 mg, 0.30 mmol) were suspended in toluene, and the suspension was stirred at 100 °C for 1 h. When the mixture was cooled, the product precipitated and was filtered off, dried, and recrystallized (acetone/water) to yield yellow crystalline **5k** (2.16 g, 94%). Mp: 196−197 °C. ^{1}H NMR (DMSO-d_6) δ 10.39 (1H, s), 9.83 (1H, s), 8.50 (1H, s), 8.17 (1H, d, J = 8.5 Hz), 8.00 (1H, s), 7.64 (1H, d, J = 7.5 Hz), 7.56 (1H, t, J = 7.5 Hz), 7.39 (1H, d, J = 7.5 Hz), 7.29 (1H, d, J = 8.5 Hz). Anal. ($C_{14}H_9ClF_3N_3O_3$) C, H, N.

1-(2-Amino-5-chlorophenyl)-3-(3-trifluoromethylphenyl)urea (5l). **5k** (1.00 g, 2.78 mmol) was dissolved in EtOH (50 mL) and treated with Raney Ni and H_2 (1 atm) under vigorous stirring for 24 h. The mixture was filtered through Celite and concentrated under vacuum. The crude product was recrystallized from EtOAc/heptane to yield red crystalline **5l** (0.8 g, 87%). Mp 181−182 °C. ^{1}H NMR (DMSO-d_6) δ 9.27 (1H, s), 8.01 (1H, s), 7.97 (1H, s), 7.52 (3H, m), 7.30 (1H, d, J = 7.5 Hz), 6.87 (1H, d, J = 8.5 Hz), 6.74 (1H, d, J = 8.5 Hz), 4.97 (2H, br s). Anal. ($C_{14}H_{11}ClF_3N_3O$) C, H, N.

3-{2-[3-(3-Bromophenyl)ureido]-4-chlorophenyl}-propionic Acid Methyl Ester (5m). 5b (600 mg, 1.46 mmol) and Pd/C (60 mg) were dissolved in EtOH (30 mL) and subjected to H_2 (3 atm) with vigorous shaking for 24 h. The solution was filtered through Celite, and the product was precipitated by addition of water, filtered off, dried, and recrystallized from EtOAc/heptane to yield colorless crystalline **5m** (435 mg, 73%). Mp: 189−190 °C. ^{1}H NMR (DMSO-d_6) δ 8.80 (1H, s), 8.73 (1H, s), 7.70 (1H, s), 7.44 (1H, d, J = 8.5 Hz), 7.25 (4H, m), 6.97 (1H, t, J = 7.5 Hz), 3.60 (3H, s), 2.89 (2H, t, J = 7.5 Hz), 2.60 (2H, t, J = 7.5 Hz). Anal. ($C_{17}H_{16}BrClN_2O_3$) C, H. N: calcd, 6.80; found, 6.34.

3-{2-[3-(3-Bromophenyl)ureido]-4-chlorophenyl}-propionic Acid (5n). 5m (300 mg, 0.73 mmol) was dissolved in 40 mL of EtOH, NaOH (4 mL, 12.5 M, 50 mmol) was added dropwise, and the mixture was stirred under N_2 for 6 h. The mixture was neutralized with 4 M HCl, and the volume was reduced to 50 mL. The product was precipitated by addition of water and recrystallized from EtOAc/heptane to yield colorless crystalline **5n** (220 mg, 76%). Mp: 193−196 °C. ^{1}H NMR (DMSO-d_6) δ 12.23 (1H, s), 8.79 (1H, s), 8.71 (1H, s), 7.69 (1H, s), 7.45 (1H, s), 7.43 (1H, s), 7.25 (3H, m), 6.97 (t, J = 7.5 Hz), 2.85 (2H, t, J = 8.0 Hz), 2.55 (2H, t. J = 8.0 Hz). Anal. ($C_{16}H_{14}BrClN_2O_3$) C, H, N.

2-[3-(3-Bromophenyl)thioureido]-4-chlorobenzoic Acid (7). 2-Amino-4-chlorobenzoic acid (**6**) (600 mg, 3.48 mmol), 3-bromophenyl isothiocyanate (800 mg, 3.73 mmol), and Et_3N (380 mg, 3.73 mmol) were dissolved in THF (10 mL), and the solution was stirred at room temperature for 6 h. The product was precipitated by adding heptane, filtered off, and recrystallized (acetone/water) to yield colorless crystalline **7** (1150 mg, 86%). Mp: 164−167 °C. ^{1}H NMR (DMSO-d_6) δ 13.70 (1H, s), 11.00 (1H, s), 10.77 (1H, s), 8.61 (1H, s), 7.91 (1H, d, J = 8.5

Hz), 7.79 (1H, s), 7.39 (3H, m), 7.24 (1H, d, J = 8.5 Hz). Anal. ($C_{14}H_{10}BrClN_2O_2S$) C, H, N.

2-(3-Bromobenzoylamino)-4-chlorobenzoic Acid (8). 2-Amino-4-chlorobenzoic acid (**6**) (1026 mg, 6.00 mmol) and 3-bromobenzoyl chloride (1.66 g, 7.20 mmol) were dissolved in dry pyridine (20 mL) at 0 °C, and the solution was stirred for 18 h. The mixture was diluted with EtOAc and washed with 1 M HCl. The organic phase was extracted with 1 M NaOH, the pH was adjusted to 2 with HCl, and the product was extracted into EtOAc. The extracts were dried with Na_2SO_4 and evaporated. The product was recrystallized from EtOAc/heptane to yield colorless crystalline **8** (830 mg, 39%). Mp: 242−244 °C (dec). ^{1}H NMR (DMSO-d_6) δ 12.28 (1H, s), 8.73 (1H, s), 8.09 (1H, s), 8.05 (1H, d, J = 8.5 Hz), 7.93 (1H, d, J = 8.0 Hz), 7.87 (1H, d, J = 8.0 Hz), 7.57 (1H, t, J = 8.0 Hz), 7.30 (1H, d, J = 8.5 Hz). Anal. ($C_{14}H_9BrClNO_3$) C, H, N.

2-[(3-Bromophenyl)carbamoyloxy]-4-chlorobenzoic Acid (10). 4-Chlorosalicylic acid (2.07 g, 12 mmol) dissolved in dry Et_2O (10 mL) was added dropwise to a solution of phosgene (7.5 mL, 20% in heptane, 15 mmol) in Et_2O (30 mL) at −20 °C. After this mixture was stirred for 30 min at −20 °C, Et_3N (2.73 g, 27.0 mmol) diluted with Et_2O (20 mL) was added dropwise over 3 h, keeping the temperature between −10 and −20 °C. The mixture was allowed to reach room temperature, stirred for 18 h, and filtered. The mother liquor was evaporated and 7-chlorobenzo[1,3]dioxine-2,4-dione (**9**) was recrystallized from Et_2O to yield a colorless crystalline solid (1450 mg, 61%). ^{1}H NMR (DMSO-d_6) δ 7.48 (1H, d, J = 8.5 Hz), 7.31 (1H, d, J = 8.5 Hz), 7.18 (1H, s). Compound **9** (700 mg, 3.53 mmol) was dissolved in THF, and 3-bromoaniline (2.00 g, 11.6 mmol) was added. The mixture was stirred for 48 h at room temperature, then diluted with EtOAc and washed with dilute HCl. The organic phase was extracted with dilute NaOH and with water. The product was precipitated from the combined aqueous phases by addition of HCl, filtered off, dried, and recrystallized from EtOAc/heptane to yield colorless crystalline **10** (840 mg, 64%). Mp: 212−215 °C. ^{1}H NMR (DMSO-d_6) δ 11.85 (1H, s), 10.43 (1H, s), 8.05 (1H, s), 7.86 (1H, d, J = 8.5 Hz), 7.63 (1H, m), 7.31 (2H, m), 7.03 (2H, m). Anal. ($C_{14}H_9BrClNO_4$) C, H, N.

2-[2-(3-Bromophenylamino)-3,4-dioxocyclobut-1-enylamino]-4-chlorobenzoic Acid (13). 3-Bromoaniline (0.86 g, 5.0 mmol) and 3,4-diethoxycyclobut-3-ene-1,2-dione (0.85 g, 5.0 mmol) were dissolved in EtOH (10 mL) and stirred at room temperature for 48 h. The product was precipitated by adding toluene, filtered off, and dried to yield crude 3-(3-bromophenylamino)-4-ethoxycyclobut-3-ene-1,2-dione (**12**) as a yellow crystalline solid. Crude **12** (0.30 g, 1.0 mmol) was dissolved in 20 mL of acetonitrile together with 2-amino-4-chlorobenzoic acid (**6**) (0.18 g, 1.0 mmol) and Et_3N (0.10 g, 1.0 mmol). The mixture was refluxed for 24 h and then concentrated to a volume of about 4 mL, and the product was precipitated by adding water (20 mL) and 4 M HCl (the pH was adjusted to 1). The product was recrystallized (EtOH/0.5 M HCl) to give colorless crystalline **13** (0.30 g, 70%). Mp: 280−290 °C (dec). ^{1}H NMR (DMSO-d_6) δ 10.91 (1H, s), 10.54 (1H, s), 7.94 (1H, d, J = 8.5 Hz), 7.79 (1H, s), 7.66 (1H, s), 7.33 (3H, m), 7.21 (1H, d, J = 8.5 Hz). Anal. ($C_{17}H_{10}BrClN_2O_4\cdot H_2O$) C, H, N.

6-Chloro-1*H*-indazol-3-ol (14). 2-Amino-4-chlorobenzoic acid (**6**) (17.16 g, 100 mmol) was suspended in 90 mL of water and 20 mL of concentrated HCl. $NaNO_2$ (6.9 g, 100 mmol) was dissolved in water (15 mL) and added slowly at 0 °C. The mixture was stirred at 0 °C for 30 min, and Na_2SO_3 (34.0 g, 270 mmol) was added, dissolved in 150 mL of water. The mixture was then stirred for 2 h at room temperature. Concentrated HCl (30 mL) was added, and the mixture was stirred overnight at room temperature and then at 80 °C for 2 h. The pH of the mixture was adjusted to 5.5 with 1 M NaOH. The precipitate was filtered off and triturated with EtOH to yield crystalline **14** (13.8 g, 82%). Mp: 292−296 °C (lit. 298−302 and 275−276 °C).[25,26] ^{1}H NMR (DMSO-d_6) δ 11.64 (1H, s), 10.58 (1H, s), 7.62 (1H, d, J = 8.5 Hz), 7.35 (1H, s), 6.97 (1H, d). Anal. ($C_7H_5ClN_2O$) C, H. N: calcd, 16.62; found, 15.89.

6-Chloro-3-hydroxyindazole-1-carboxylic Acid (3-Bromophenyl)amide (15). Indazolol **14** (700 mg, 4.15 mmol) was dissolved in 25 mL of pyridine, together with 3-bromophenyl isocyanate (990 mg, 5 mmol). The mixture was stirred at room temperature for 4 h, then diluted with EtOAc (100 mL) and washed twice with 1 M HCl. The organic phase was dried with Na_2SO_4 and evaporated under vacuum. The residue was dissolved in 1 M NaOH and washed with EtOAc, and the product was precipitated with HCl. The precipitate was extracted into EtOAc, and the solvent was removed under vacuum. The crude product was recrystallized twice from EtOAc/heptane to yield colorless crystalline **15** (770 mg 55%). Mp: 223−225 °C. ^{1}H NMR (DMSO-d_6) δ 12.20 (1H, s), 10.01 (1H, s), 8.23 (1H, s), 8.06 (1H, s), 7.95 (1H, d, J = 8.5 Hz), 7.72 (1H, d, J = 7.5 Hz), 7.37 (1H, d, J = 8.5 Hz), 7.30 (2H, m). Anal. ($C_{14}H_9BrClN_3O_2$) C, H, N.

Stable Cell Lines. HEK293 cell lines stably expressing homomeric human GluR5−1a and human GluR6 were established as described previously.[27] HEK293 cell lines stably expressing homomeric rat GluR1−4 were established using a modified version of the bicistronic expression vector pIRES (ClonTech, Palo Alto, CA), pIRES-BLAS-AN,[28] using blasticidin selection for incorporation of the flip isoforms of rat GluR1−4. Cell lines were grown in Dulbecco's modified Eagle's medium supplemented with 10% (v/v) fetal calf serum in polystyrene culture flasks (175 cm^2) in a humidified atmosphere of 5% CO_2 and 95% O_2 at 37 °C. Growth media for GluR1−4 cell lines were furthermore supplemented with 100 μg/mL SPD 502, an AMPA receptor antagonist.[27,29] Cells were cultured to 80−90% confluency before plating. The cells were rinsed with 10 mL of phosphate buffered saline (PBS), 1.5 mL of trypsin−EDTA (0.1% (w/v) trypsin) was added, and the cells were left in the incubator for 5 min. After addition of 10 mL of growth media, cells were resuspended by trituration with a 10 mL pipet. The cells were seeded at a density of (0.5−5) × 10^6 cells/mL (100 μL/well) in black-walled, clear-bottom 96-well plates pretreated with 0.001% (w/v) polycation polyethyleneimine (PEI) solution (75 μL/well for ≥30 min). Plated cells were allowed to proliferate for 24 h before loading with dye.

Fluorescence Measurements. On the day of experiment the medium was removed from the wells, and 50 μL of the Fluo-4-AM (cell-permeant acetoxymethyl ester of the Ca^{2+} indicator Fluo-4; Molecular Probes) loading solution (2 μM in medium) was added to each well. The plates were sealed and incubated at room temperature for 60 min (GluR5,6) or 30 min (GluR1−4). After the loading period, the loading media was aspirated and the cells were washed twice with 100 μL of Na^+-free Ringer (10 mM HEPES, 140 mM choline chloride, 5 mM KCl, 1 mM $MgCl_2$, 10 mM $CaCl_2$; pH 7.4) to remove extracellular dye. Na^+-free Ringer (100 μL) was added to each well, and the fluorescence was measured at room temperature (excitation 488 nm, emission 510−570 nm band-pass interference filter) in the FLIPR (Molecular Devices, Sunnyvale, CA). Cells were preincubated for 1.5 min with test compound (50 μL) before addition of agonist (50 μL) to a final concentration of 2 μM domoic acid (for GluR5), 0.2 μM domoic acid (for GluR6), or 25 μM Glu (for GluR1−4). For GluR1−4, all incubation solutions contained 100 μM cyclothiazide to inhibit receptor desensitization. Stock solutions of test substances were prepared in EtOH or DMSO, with the final concentration of these solvents never exceeding 1.0% in the prepared test solutions.

Ligand Binding Studies. GluR5-expressing cells were harvested, washed once with 50 mM Tris-HCl (pH 7.1), and stored at −80 °C until the day of experiment. The thawed membrane pellets were resuspended in >100 volumes of ice-cold Tris-HCl buffer and centrifuged at 27000*g* for 10 min. The final pellet was resuspended in Tris-HCl buffer and used for binding experiments. All procedures were performed at 0−4 °C.

Binding conditions for GluR5 were as described previously.[27] Briefly, binding to GluR5 receptors was performed using 3 nM [^{3}H]ATPA and 46−84 μg of protein/assay. The samples were

incubated in a final volume of 550 μL for 60 min at 2 °C. Nonspecific binding was determined in the presence of 0.6 mM Glu, and binding was terminated by rapid filtration. Radioactivity was determined by conventional liquid scintillation counting.

ATPA-Induced Rigidity. Rigidity in mice was induced by intravenous administration of ATPA. ATPA is systemically active[30] and has agonistic activity primarily at the GluR5 subtype.[31,32] Intravenous administration of ATPA in 20−25 g mice (female NMRI, Taconic M&B, Denmark) produces a characteristic muscle rigidity that can be blocked by KA/AMPA receptor antagonists.[33,34] ATPA was dissolved in water by addition of one drop of 1 M NaOH, addition of saline, and titration back to pH 7 (3 mg/mL). Test substances were administrated intravenously ($n = 8$ per dose) 5 or 30 min prior to the ATPA (30 mg/kg) administration. Rigidity was scored by the number of mice showing loss of righting and lack of movement on 45° grid grib 30 min after ATPA administration. The data are presented as the percent of mice for each experimental group showing rigidity.

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Supporting Information Available: Results from elemental analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

Note Added after ASAP Publication. This manuscript was released ASAP on 12/2/2004 with errors in the artwork and footnotes of Scheme 2 and in the listing of the starting material for the preparation of **10** (in Results (second paragraph) and in the Experimental Section). The correct version was posted on 12/8/2004.

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