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

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A series of 2-arylureidobenzoic acids (AUBAs) was prepared by a short and effective synthesis, and the pharmacological activity at glutamate receptors was evaluated in vitro and in vivo. The compounds showed noncompetitive antagonistic activity at the kainate receptor subtype GluR5. The most potent compounds showed more than 50-fold selectivity for GluR5 compared to GluR6 and the AMPA receptor subtypes GluR1−4. The structure−activity relationships for the AUBAs showed distinct structural requirements for the substituents on the two aromatic ring systems. Only para-substituents were tolerated on the benzoic acid moiety (ring A), whereas ring B tolerated a variety of substituents, but with a preference for lipophilic substituents. The most potent compounds had a 4-chloro substituent on ring A and 3-chlorobenzene (**6b**), 2-naphthalene (**8h**), or 2-indole (**8k**) as ring B and had IC_{50} values of 1.3, 1.2, and 1.2 μM, respectively, in a functional GluR5 assay. Compound **6c** (IC_{50} = 4.8 μM at GluR5) showed activity in the in vivo ATPA rigidity test, indicating that **6c** has better pharmacokinetic properties than **8h**, which was inactive in this test. The AUBAs are the first example of a series of noncompetitive GluR5-selective antagonists and may prove to be important pharmacological tools and leads in the search for therapeutic glutamatergic agents.

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 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, -B), four AMPA subunits (GluR1−4), and five KA subunits (GluR5−7, KA1, and -2) 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 the GluR5 and the 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]

The development of (3*S*,4a*R*,6*R*,8a*R*)-6-[2-(1*H*-tetrazol-5-yl)ethyl]-decahydroisoquinoline-3-carboxylic acid (LY 293558), 6-(4-carboxybenzyl)-decahydroisoquinoline-3-carboxylic acid (LY 382884) (Figure 1), and other decahydroisoquinolines as the first GluR5-selective antagonists[10,11] has enabled researchers to gain insight into the physiological role of GluR5 and suggested this subunit as a potential therapeutic target.[5−11] All the aforementioned GluR5-selective antagonists are competitive, but several noncompetitive AMPA receptor antagonists have been reported, e.g. 1-(4-aminophenyl)-4-methyl-7,8-methylenedioxy-5*H*-2,3-benzodiazepine (GYKI 52466) and 1-(4-aminophenyl)-3-methylcarbamoyl-4-methyl-3,4-dihydro-7,8-methylenedioxy-5*H*-2,3-benzodiazepine (LY 300168) (Figure 1).[11] Because noncompetitive antagonists elicit their receptor blockade regardless of the endogenous excitatory ligand concentration, these agents can modulate neurotransmission in a manner different from that of competitive agents; thus, from a therapeutic perspective noncompetitive agents may potentially be more efficacious than competitive agents.[12]

We present a new class of Glu antagonists, 2-arylureidobenzoic acids (AUBAs, **1**−**8**), which are noncompetitive and GluR5-selective. We know of only one other noncompetitive GluR5-selective antagonist, namely, 5-carboxyl-2,4-dibenzamido-benzoic acid (NS3763, IC_{50} = 1.6 μM) (Figure 1), which was discovered recently and disclosed in a manuscript that has been submitted for

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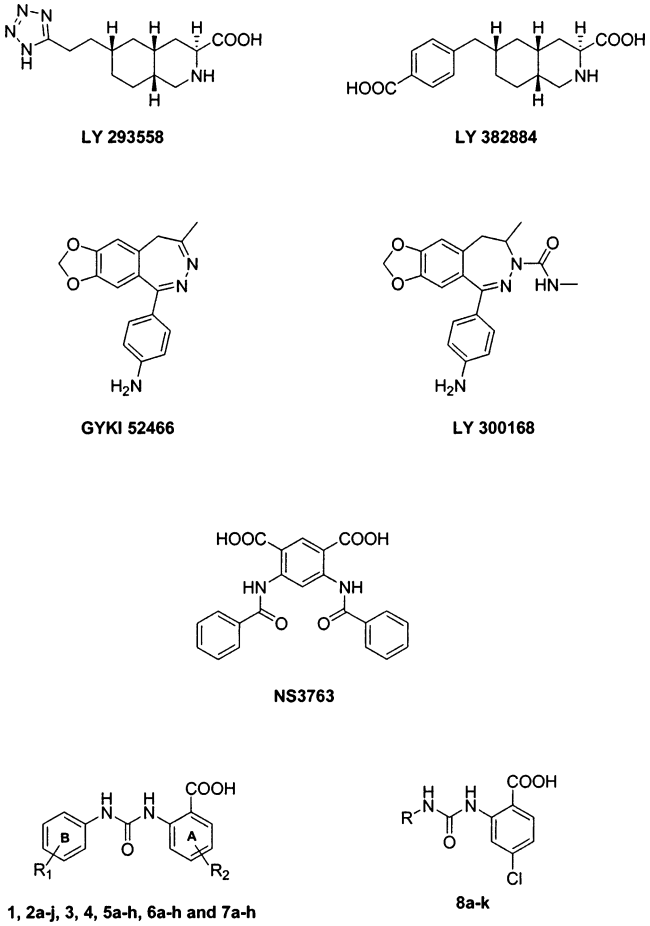


Figure 1. Structures of the competitive GluR5-selective antagonists LY 293558 and LY 382884, the noncompetitive AMPA antagonists GYKI 54266 and LY 300168, the noncompetitive GluR5 antagonist NS3763, and generalized structures of the new series of noncompetitive GluR5 antagonists, the 2-arylureidobenzoic acids (See Tables 1−3 for lists of substituents).

publication.[13] The AUBAs are therefore the first series of noncompetitive GluR5-selective antagonists to be published. The structure−activity relationships are studied and these compounds may be valuable tools for pharmacological studies of GluR5 and the development of GluR5 antagonists as therapeutic agents.

Results

The AUBAs **1**, **2a**−**j**, **3**, **4**, **5a**−**h**, **6a**−**h**, **7a**−**h**, and **8a**−**k** were synthesized by condensing the appropriate isocyanates (**11a**−**v** and **14a**−**k**) with anthranilic acids (**12a**−**k**), as shown in Scheme 1. These reactions usually proceeded smoothly in polar aprotic solvents such as THF or DMSO at room temperature. Stronger conditions, such as the use of Et_3N and higher temperatures, increased the speed of the reaction but decreased the yields, because dimerization of the isocyanates and cyclization of the desired products into side products of type **15** and **16** (Figure 2), respectively, became pronounced. Those isocyanates that were not commercially available were synthesized from the appropriate benzoic acids (**9a**−**b** and **13a**−**h**) via the Curtius rearrangement using diphenylphosphoryl azide and reacted further with the appropriate anthranilic acid in one pot. All the anthranilic acids were commercially available except 4-trifluoromethylanthranilic acid (**12i**), which was prepared from 2-nitro-4-trifluoromethylbenzoic acid **10** by catalytic hydrogenation.

The hydroxy-substituted analogues **5**−**7h** were obtained from the methoxy-substituted compounds **5**−**7g** by demethylation with BBr_3 in CH_2Cl_2 (Scheme 1). The 3-biphenyl analogue **6f** was prepared from 3-phenylbenzoic acid **9b** (R = 3-Ph−Ph), which was prepared from 3-iodobenzoic acid by a Pd-catalyzed cross-coupling with phenylboronic acid. Compound **9b** was transformed into isocyanate **11v** by the Curtius rearrangement and subsequently condensed with 4-chloroanthranilic acid **12g** to yield **6f**.

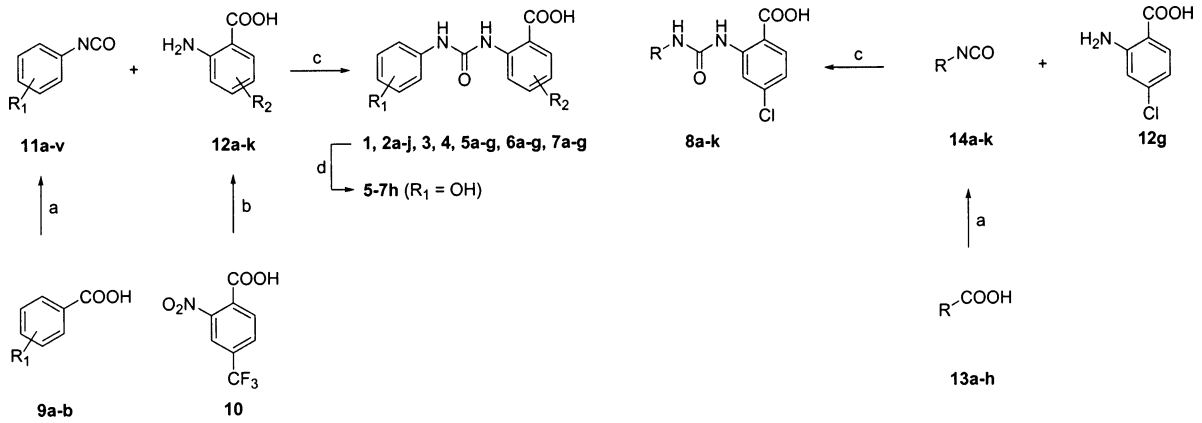
In Vitro Pharmacology. The antagonistic effects of the compounds on the different recombinant homomeric rat AMPA and human KA receptors (GluR1−6), stably expressed in HEK293 cells were characterized by functional assays using FLIPR (fluorescent imaging plate reader) technology. The antagonistic activity 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 domoate for GluR5−6) to cells expressing the appropriate receptor.

The IC_{50} values determined at GluR5 are presented in Tables 1−3. The benzoic acid moiety is designated as ring A and the other aromatic system as ring B.

The unsubstituted phenylureidobenzoic acid **1** was inactive at GluR5 (IC_{50} >100 μM), but the introduction of a trifluoromethyl substituent in position 3 on ring B as in **2a** resulted in a measurable, but weak, activity (Table 1). Using **2a** as a lead structure, keeping the CF_3 substituent on ring B constant, and introducing methyl groups into positions 3, 4, 5, or 6 on ring A showed that methyl groups in position 3, 5, and, 6 (**2b**, **2d** and **2e**, respectively) abolished activity completely, whereas the 4-methylated compound **2c** was twice as potent as the unmethylated parent compound **2a**. Exploring position 4 in ring A further resulted in compounds **2f**−**j** and **3**, revealing the 4-chloro-substituted compound (**2g**) to be six times more potent (IC_{50} = 5.0 μM) than the parent compound (**2c**). The other halogens, Br and F, did also markedly increase the activity, whereas NO_2 and CF_3 did so only marginally. The naphthalene analogue **3** was distinctly less potent.

Changing ring B systematically, while keeping ring A optimally substituted with the 4-chloro substituent, yielded the compounds presented in Tables 2 and 3. Introduction of halogens or trifluoromethyl groups increased the activity about 2−3-fold in all positions compared to **4**. The only exception was the introduction of fluoro substituents, which had insignificant effects in positions 3 and 4, but resulted in 2-fold reduction in activity in position 2 (Table 2). The introduction of biphenyls as ring B as in compounds **5**−**7f** did not result in increased potency compared to **4**. The hydrogen-bond-accepting methoxy group had insignificant effects in positions 2 and 4, but increased the potency by a factor of 3 in position 3. The hydrophilic hydroxy group markedly decreased the potency in position 2 and 4 but only slightly in position 3. The 3-chloro derivative **6b** was the most potent (IC_{50} = 1.3 μM) of the compounds that had a substituted phenyl ring as ring B. The phenyl ring B of **4** was replaced by various other cyclic systems

Scheme 1[a]



[a] Reagents and conditions: (a) diphenylphosphoryl azide, Et_3N, toluene, 80 °C; (b) H_2 (1 atm), Pd/C, EtOH, rt; (c) THF, rt; (d) BBr_3, CH_2Cl_2, rt. See Tables 1−3 for a list of substituents.

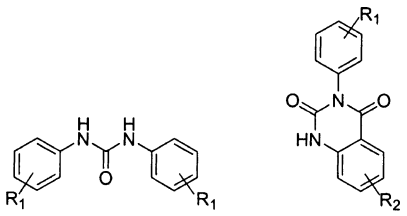
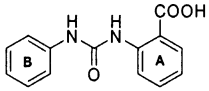
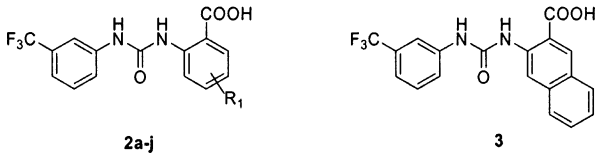


Figure 2. Byproducts **15** and **16**, from the condensation of isocyanates and anthranilic acids, formed when vigorous conditions were employed (see text).

Table 1. Inhibition of Agonist-Induced GluR5 Responses by Compounds Bearing Different Substituents on Ring A[a]



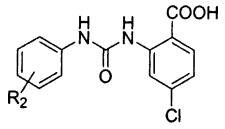


compd	R_1	IC_{50} (μM)	compd	R_1	IC_{50} (μM)
1		>100	**2f**	4-F	10 ± 1.0
2a	H	72 ± 18	**2g**	4-Cl	5.0 ± 1.1
2b	3-Me	>100	**2h**	4-Br	6.1 ± 1.6
2c	4-Me	31 ± 10	**2i**	4-CF_3	21 ± 2.5
2d	5-Me	>100	**2j**	4-NO_2	30 ± 6.9
2e	6-Me	>100	**3**		60 ± 2.8

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

to yield the compounds presented in Table 3. The saturated analogue **8a** was inactive (IC_{50} > 100 μM). Pyridines **8b**−**d** were 3−9-fold less potent than the phenyl compound **4**, and the thiophenes **8e**,**f** were 2−5 times less potent than **4**. The 2-naphthyl derivative **8h** and 2-indolyl derivative **8k** were the two most potent compounds of the whole series with similar IC_{50} values of 1.2 μM, which is approximately 8 times more potent than **4**. The 1-naphthyl derivative **8g** was, however, much less potent (IC_{50} = 9.4 μM) than its 2-substituted analogue, and the anthracenyl compound **8i** was inactive at the concentration tested. The 5-benzo[1,3]dioxolyl derivative **8j** was about twice as potent as **4**.

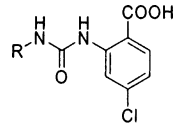
Table 2. Inhibition of Agonist-Induced GluR5 Responses by Compounds Bearing Different Substituents on Ring B[a]



compd	R_2	IC_{50} (μM)	compd	R_2	IC_{50} (μM)
4	H	10 ± 2.7	**6e** = **2g**	3-CF_3	5.0 ± 1.1
5a	2-F	20 ± 6.0	**6f**	3-phenyl	12 ± 1.7
5b	2-Cl	4.7 ± 1.6	**6g**	3-OMe	3.7 ± 0.51
5c	2-Br	4.5 ± 1.2	**6h**	3-OH	14 ± 2.5
5d	2-I	5.0 ± 1.5	**7a**	4-F	14 ± 1.8
5e	2-CF_3	4.5 ± 1.2	**7b**	4-Cl	3.1 ± 0.47
5f	2-phenyl	>10	**7c**	4-Br	2.2 ± 0.35
5g	2-OMe	12 ± 1.7	**7d**	4-I	4.7 ± 0.46
5h	2-OH	21 ± 3.9	**7e**	4-CF_3	4.8 ± 0.85
6a	3-F	7.6 ± 2.7	**7f**	4-phenyl	29 ± 5.0
6b	3-Cl	1.3 ± 0.49	**7g**	4-OMe	10 ± 2.7
6c	3-Br	4.8 ± 1.1	**7h**	4-OH	36 ± 4.7
6d	3-I	2.2 ± 0.66			

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

Table 3. Inhibition of Agonist-Induced GluR5 Responses by Compounds Where Phenyl Ring B Has Been Exchanged for Different Heterocyclic and Bicyclic Systems[a]



compd	R	IC_{50} (μM)	compd	R	IC_{50} (μM)
8a	cyclohexyl	>100	**8g**	1-naphthyl	9.4 ± 2.8
8b	2-pyridyl	31 ± 11	**8h**	2-naphthyl	1.2 ± 0.35
8c	3-pyridyl	69 ± 17	**8i**	2-anthracenyl	>30
8d	4-pyridyl	88 ± 6.9	**8j**	5-benzo[1,3]dioxolyl	3.6 ± 0.85
8e	2-thienyl	18 ± 3.9	**8k**	2-indolyl	1.2 ± 0.37
8f	3-thienyl	51 ± 17			

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

All of the AUBAs except **6c** had IC_{50} values higher than 30 μM in the GluR6 assay. With this background, **6c** was chosen for further pharmacological characterization together with the most potent analogue **8h** (Table 4). Compounds **6c** and **8h** did not show any GluR1−4 antagonistic activity at 30 μM and only weak inhibition (less than 50%) at 100 μM. The IC_{50} values of the two

Table 4. Potencies of Selected Compounds in Functional Assays for GluR1−6 (FLIPR assays and electrophysiology on GluR5), [^{3}H]ATPA Binding (all as IC_{50} in μM)[a] and ATPA Rigidity (as ED_{50} in mg/kg)

	antagonism in FLIPR assay			GluR5		ATPA rigidity	
compd	GluR1−4	GluR5	GluR6	electrophysiology	[^{3}H]ATPA binding	5 min	30 min
6c	>100	4.8 ± 1.1	28 ± 6.9	3.2	>30	4	24
8h	>100	1.2 ± 0.35	62 ± 10		>30	>30	>30

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

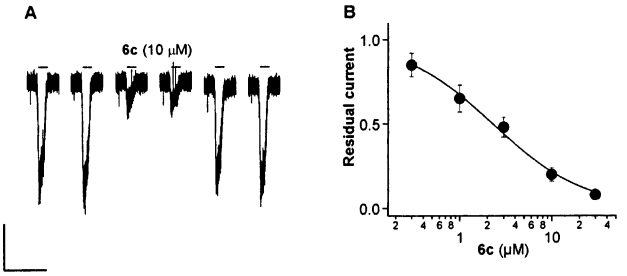


Figure 3. Electrophysiological characterization of **6c**. (A) Representative current trace showing the inhibition by 10 μM **6c** of responses evoked by 1 mM Glu from HEK293 cells expressing GluR5 receptors. Scale bars: 100 pA/5 s. (B) Concentration−response curve for the effect of **6c**. Data points represent means ± SEM (n = 3). Curve fitting was performed as described in the Experimental Section.

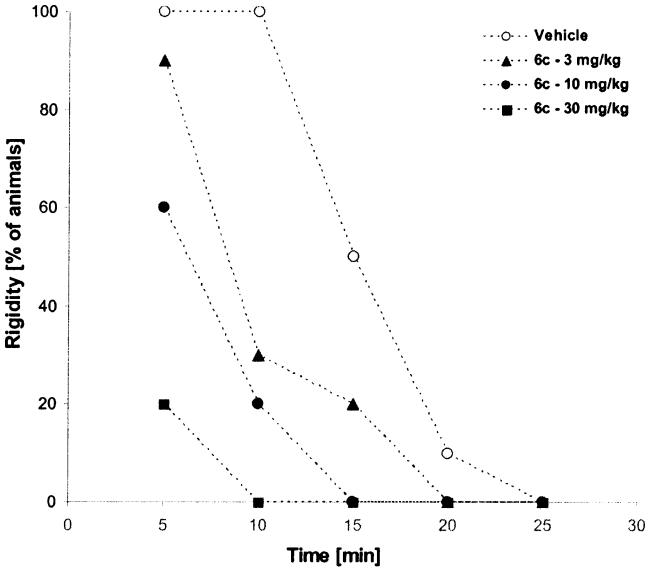


Figure 4. Example of data obtained from the ATPA rigidity test. The graph shows how the administration of different doses of **6c**, 5 min prior to the administration of ATPA, inhibited the rigidity (expressed as the percentage of rigid animals) induced by ATPA in a dose-dependent manner.

at GluR6 were approximately 6 and 50 times higher, respectively, than at GluR5.

Binding studies using recombinant receptors and the GluR5-selective agonist [^{3}H](*RS*)-2-amino-3-(3-hydroxy-5-*tert*-butyl-4-isoxazolylpropionic acid ([^{3}H]ATPA) were performed for compounds **6c** and **8h** and showed that they did not inhibit [^{3}H]ATPA binding to GluR5 receptors at the concentrations tested (3−30 μM).

Electrophysiology. The effect of compound **6c** on HEK293 cells expressing GluR5 was measured using patch-clamp electrophysiology. Compound **6c** reversibly inhibited the currents induced by Glu in a concentration-dependent manner (Figure 3) with an IC_{50} of 3.2 μM, which is comparable to its potency in the FLIPR assay (IC_{50} = 4.8 μM).

In Vivo Pharmacology. The in vivo activities of compounds **6c** and **8h** were evaluated in the ATPA-induced rigidity test in mice. The test compounds were administered prior to the administration of the GluR5 agonist ATPA and the degree of rigidity induced by ATPA was observed. Compound **6c** inhibited the rigidity with an ED_{50} value of 4 mg/kg when ATPA was administered 5 min after compound **6c**. The ED_{50} value increased to 24 mg/kg when the delay between the two administrations was extended to 30 min (Table 4 and Figure 4). Compound **8h** did not affect the rigidity at the doses tested (10 and 30 mg/kg).

QSAR Analysis. QSAR analysis was performed to establish possible correlations (correlation analysis calculated with Sigmaplot 8.0, SPSS, Chicago, IL) between the observed inhibitory activity at GluR5 and different physicochemical properties of the compounds. No significant correlation was found between the observed GluR5 activity and lipophilicity, electronic, or steric properties of the different substituents in position 4 on ring A. However, a correlation was found between the calculated log P (log P was calculated with Chemdraw Ultra 7.0, Cambridgesoft, Cambridge, MA) of the ring B substituted compounds **2g**, **4**, **5a**−**h**, **6a**−**h**, **7a**−**h**, and **8b**−**d** and their activity at GluR5 (eqs 1−4 and Figure 5). The correlation was most pronounced when the compounds were grouped by the site of substitution, and the correlation was strongest for the ortho (eq 1) and para (eq 3) series (r^2 = 0.89 and 0.83, respectively), but weaker for the meta series (eq 2, r^2 = 0.62). When the whole series was treated as one group, the r^2 was 0.72 (eq 4).

$$\log IC_{50} = -0.70 (\log P) + 4.1 \quad \text{(ortho series)} \quad (1)$$

$$r^2 = 0.89$$

$$\log IC_{50} = -0.53 (\log P) + 3.4 \quad \text{(meta series)} \quad (2)$$

$$r^2 = 0.62$$

$$\log IC_{50} = -0.53 (\log P) + 3.5 \quad \text{(para series)} \quad (3)$$

$$r^2 = 0.83$$

$$\log IC_{50} = -0.60 (\log P) + 3.8 \quad \text{(whole series)} \quad (4)$$

$$r^2 = 0.72$$

The biphenyl analogues **5**−**7f** were left out of the correlation calculations, as they were outliers (open circles in Figure 5). This was expected because the phenyl substituent is much larger than the other substituents and no size parameter was included in the QSAR equations. Similar correlations were seen when the substituent lipophilicity parameter π was used instead of log P (data not shown).

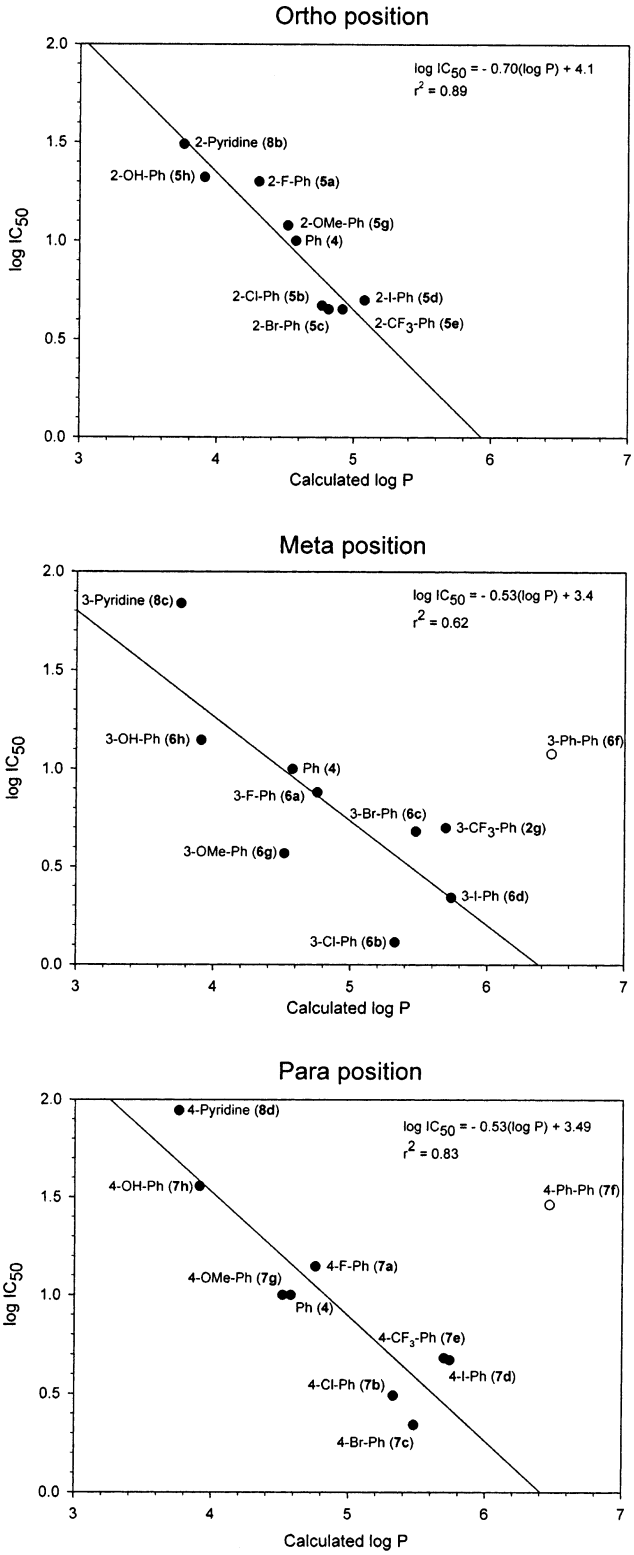


Figure 5. Plot of IC_{50} values (μM) at GluR5 versus calculated log *P* values for ortho-, meta-, and para-substituted phenyls and unsubstituted 1-, 2-, and 3-pyridyls as ring B.

Discussion

A short and convenient synthesis of the AUBA GluR5 antagonists was developed. The Curtius rearrangement and subsequent condensation with anthranilic acids were adapted for one-pot synthesis using a carousel reaction station, enabling parallel synthesis of multiple compounds.

The structure−activity relationship in this series of noncompetitive GluR5 antagonists presents a picture of a binding site where rather strict structural requirements apply around ring A. Thus, compounds with methyl substituents in positions 3, 5, and 6 on ring A (**2b**, **2d** and **2e**) had no measurable activity, but the 4-methylated analogue **2c** was active. The choice of substituents in position 4 also had profound effect on the potency. Initially, it seemed plausible that the increased activity of the 4-halogenated analogues, e.g. **2g** and **2h**, compared to the 4-methyl analogue **2c**, was due to increased acidity of the benzoic acid functionality, caused by the electron-withdrawing effects of the halogens. The 4-trifluoromethyl and 4-nitro analogues **2i** and **2j** did, however, not support this hypothesis. Even though these substituents are very electron-withdrawing, the two analogues possessing these substituents showed low potency. This indicates that the effect is more complex in nature and that the chloro and bromo substituents have optimal properties for interacting with the receptor. The low potency of the naphthalene analogue **3** also implies that steric limitations apply around ring A.

When comparing the activity of compounds substituted on ring B (shown in Tables 2 and 3), there is a preference for meta-substituted compounds over ortho- and para-substituted compounds. The activity of the meta-substituted compounds could not be correlated with their lipophilicity to the same degree as the ortho- and para-substituted compounds ($r^2 = 0.62$ for meta, versus 0.89 and 0.83 for ortho and para, respectively), perhaps indicating a specific interaction with the receptor at the meta position. The observation that the 2-naphthyl compound **8h** is 8 times more potent than the 1-naphthyl analogue **8g** also indicates preference toward the meta/para positions over the ortho position. The 2-indolyl compound **8k** is also potent and it has a similar linear shape as **8h**, whereas **8g** has a more angular shape (assuming that the urea functionality has syn/syn conformation in all cases), further establishing that linear bicyclics and meta-substituted phenyls are preferred over other isomers.

QSAR analysis showed that the lipophilicity of the substituents around ring B is a determining factor for activity. The low potency of the heterocyclic analogues **8b**−**f** may be attributed to the low lipophilicity of these compounds compared to **4**. Lipophilic substituents can increase activity through two mechanisms: in a specific manner, through interactions with a lipophilic pocket around ring B, or in a nonspecific manner, by interfering with the solvation of the ligand in the aqueous environment outside the receptor (make the free energy of solvation less negative) and thereby inherently increase the binding affinity of the compounds to all receptors. We favor the latter mechanism as an explanation of the observed structure−activity relationship around ring B, due to the following: The choice of substituents and different sites of substitution on ring B did not have the drastic effect on activity one would expect if there was close contact with the receptor protein around this ring. Furthermore, large substituents, such as 1-naphthyl and the biphenyls, did not eliminate activity completely but only reduced the potency; thus, these compound can to some degree be accommodated by the receptor despite

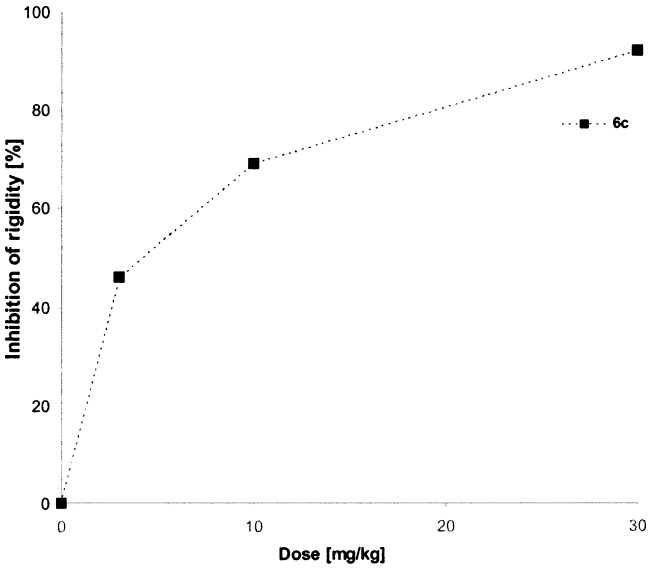


Figure 6. Example of a dose−response curve obtained in the ATPA rigidity test. The inhibition of rigidity was derived by calculating the area under the curve (AUC) for each dose in Figure 4 compared to the AUC for the vehicle alone (inhibition of rigidity = $[1 - AUC_{dose}/AUC_{vehicle}] \times 100\%$).

their bulk. These large compounds are, however, very lipophilic and if they should follow the same QSAR that was observed for the substituted phenyls in Table 2, they should be much more potent than they turned out to be.

Compound **8h** was, together with **8k**, the most potent GluR5 antagonist. However, compound **8h** did not inhibit ATPA-induced rigidity, whereas **6c** did, with an ED_{50} of 4 mg/kg (Figure 6). As **8h** was 4 times more potent than **6c** in vitro, we attribute the lack of effect observed for **8h** in the ATPA rigidity test to pharmacokinetic problems. Compared to **6c**, **8h** is more lipophilic and it may be bound to proteins to a larger extent, or it may be metabolized fast. The antagonistic effect of compound **6c** was further validated in electrophysiological studies using cells expressing GluR5 receptors. The location of the AUBA binding site is unknown, but the compounds are noncompetitive antagonists, as they do not compete with the orthosteric ligand ATPA.

Conclusion

The AUBAs represent a new class of conveniently synthesized GluR5-selective antagonists, which have shown activity both in vitro and in vivo. Their in vitro potency is comparable with that of NS3763 (IC_{50} = 1.6 μM) (Figure 1), which is the only other noncompetitive GluR5-selective antagonist known.[13] The classical noncompetitive AMPA antagonists, e.g. GYKI 52466 and LY 300168 (Figure 1), also have IC_{50} values in the low micromolar range (for AMPA receptors).[11] The most potent compound in this series in vitro is **8h**, which has an IC_{50} of 1.2 μM for GluR5. Furthermore, compound **8h** is selective with IC_{50} values for GluR1−4 and GluR6 being at least 50 times higher than for GluR5. The in vitro pharmacology of the whole series reveals a structure−activity relationship that is very sensitive to the substituents around ring A but is much more flexible around ring B, allowing considerable variation of substituents and ring types at that site. Compound **6c**, which was 4 times less potent than **8h** in vitro, did, however, perform much better in vivo in the ATPA-induced rigidity test, implying that pharmacokinetic factors may play a significant role in the in vivo activity of these compounds. Investigations using carboxylic acid bioisosteres and modification of the urea linker to further modulate the activity and the pharmacokinetics of these compounds are underway in our laboratory.

Experimental Section

Chemistry. All reagents are commercially available unless stated otherwise. All reactions were carried out under a 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 1, 2a−h,j, 3, 4, 5a−c,e−g, 6a−d,g, 7a−g, and 8g,j. The appropriate isocyanate (2.1 mmol) and appropriate anthranilic acid (2.0 mmol) were dissolved/suspended in dry THF (10 mL), toluene (100 mL), or DMSO (10 mL) and stirred at room temperature until one or both starting materials could not be detected by TLC (typically 2−3 h when run in THF or 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 for each compound.

General Procedure B: Synthesis of AUBAs 5d, 6f, and 8b−f,h,i,k. The appropriate carboxylic acid (**9a**,**b** or **13a**−**h**) (3.0 mmol) was suspended in dry toluene (15 mL), and diphenylphosphoryl azide (958 mg, 3.5 mmol) was added. Triethylamine (354 mg, 3.6 mmol) was then added dropwise and the mixture stirred at room temperature for 30 min. The solution was heated to 80 °C for 2 h. 2-Amino-4-chlorobenzoic acid (**12g**) (460 mg, 2.7 mmol) in THF (20 mL) was added together with triethylamine (0.35 g, 3.6 mmol). The mixture was stirred for 1 h at 80 °C and then at room temperature overnight. The reaction mixture was diluted with 200 mL of EtOAc and then washed with 1 M HCl and with saturated sodium bicarbonate. The organic phase was then extracted three times with water. The title compound was precipitated from the combined aqueous phases by addition of HCl. The product was filtered off, washed, and recrystallized as described for each compound.

General Procedure C: Synthesis of AUBAs 5−7h. The appropriate 4-chloro-2-[(methoxyphenyl)ureido]benzoic acid **5**−**7g** (1.5 mmol) was suspended in 100 mL of CH_2Cl_2 at 0 °C and BBr_3 (1.8 mL, 1.0 M in hexane, 1.8 mmol) was added. The mixture was stirred overnight and then more BBr_3 (0.9 mL, 1.0 M in hexane, 0.9 mmol) was added and the mixture stirred overnight. The mixture was diluted with EtOAc, washed with water, dried with Na_2SO_4, and evaporated under vacuum and recrystallized as described for each compound.

2-(3-Phenylureido)benzoic acid (1) was prepared according to general procedure A. The crude product was dissolved in 0.5 M NaOH and precipitated by addition of 4 M HCl. Yield: 80%. Mp: 168−169 °C. ^{1}H NMR (DMSO-d_6): δ 13.47 (1H, br s), 10.36 (1H, s), 9.80 (1H, s), 8.36 (1H, d, J = 8 Hz), 7.95 (1H, d, J = 8 Hz), 7.53 (3H, m), 7.28 (2H, t, J = 8 Hz), 7.03 (1H, t, J = 8 Hz), 6.98 (1H, t, J = 8 Hz). Anal. ($C_{14}H_{12}N_2O_3$) C, H, N.

2-[3-(3-Trifluoromethylphenyl)ureido]benzoic acid (2a) was prepared according to general procedure A. The crude product was dissolved in 0.5 M NaOH and precipitated by addition of 4 M HCl. Yield: 93%. Mp: 171−172 °C. ^{1}H NMR (DMSO-d_6): δ 13.50 (1H, br s), 10.51 (1H, s), 10.21 (1H, s), 8.39 (1H, d, J = 8.3 Hz), 8.00 (1H, s), 7.97 (1H, d, J = 8.3 Hz), 7.72 (1H, d, J = 7.7 Hz), 7.55 (1H, t, J = 8.3 Hz), 7.51 (1H, t, J = 7.7 Hz), 7.30 (1H, d, J = 7.7 Hz), 7.05 (1H, t, J = 8.3 Hz). Anal. ($C_{15}H_{11}F_3N_2O_3$) C, H, N.

3-Methyl-2-[3-(3-trifluoromethylphenyl)ureido]-benzoic acid (2b) was prepared according to general procedure A. The crude product was dissolved in 0.5 M NaOH and precipitated by addition of 4 M HCl. Yield: 83%. Mp: 165−166 °C. ^{1}H NMR (DMSO-d_6): δ 13.06 (1H, br s), 9.73 (1H, s), 8.76 (1H, s), 8.00 (1H, s), 7.67 (1H, d, J = 7.4 Hz), 7.58 (1H, d, J = 7.8 Hz), 7.51 (1H, t, J = 7.4 Hz), 7.44 (1H, d, J = 7.8 Hz) 7.28 (1H, d, J = 7.4 Hz), 7.19 (1H, t, J = 7.8 Hz), 2.25 (3H, s). Anal. ($C_{16}H_{13}F_3N_2O_3 \cdot {}^1/_4H_2O$) C, H, N.

4-Methyl-2-[3-(3-trifluoromethylphenyl)ureido]-benzoic acid (2c) was prepared according to general procedure A. The product was recrystallized (EtOAc−heptane). Yield: 87%. Mp: 179−182 °C. ^{1}H NMR (DMSO-d_6): δ 13.32 (1H, br s), 10.52 (1H, s), 10.17 (1H, s), 8.25 (1H, s), 8.05 (1H, s), 7.86 (1H, d, J = 7.8 Hz), 7.69 (1H, d, J = 8.3 Hz), 7.52 (1H, t, J = 7.8 Hz), 7.32 (1H, d, J = 7.8 Hz), 6.89 (1H, d, J = 8.3 Hz), 2.34 (3H, s). Anal. ($C_{16}H_{13}F_3N_2O_3$) C, H, N.

5-Methyl-2-[3-(3-trifluoromethylphenyl)ureido]-benzoic acid (2d) was prepared according to general procedure A. The product was recrystallized (MeOH−H_2O). Yield: 72%. Mp: 183−184 °C (dec). ^{1}H NMR (DMSO-d_6): δ 13.45 (1H, br s), 10.37 (1H, s), 10.13 (1H, s), 8.27 (1H, d, J = 8.6 Hz), 8.02 (1H, s), 7.77 (1H, s), 7.70 (1H, d, J = 7.9 Hz), 7.51 (1H, t, J = 7.9 Hz), 7.37 (1H, d, J = 8.6 Hz), 7.31 (1H, d, J = 7.9 Hz), 2.28 (3H, s). Anal. ($C_{16}H_{13}F_3N_2O_3$) C, H, N.

6-Methyl-2-[3-(3-trifluoromethylphenyl)ureido]-benzoic acid (2e) was prepared according to general procedure A. The crude product was dissolved in 0.5 M NaOH and precipitated by addition of 4 M HCl. Yield: 68%. Mp: 181−182 °C. ^{1}H NMR (DMSO-d_6): δ 13.55 (1H, br s), 9.86 (1H, s), 8.64 (1H, s), 8.00 (1H, s), 7.72 (1H, d, J = 8.3 Hz), 7.56 (1H, d, J = 7.6 Hz), 7.51 (1H, t, J = 8.1 Hz), 7.30 (1H, t, J = 7.6 Hz), 7.27 (1H, d, J = 8.1 Hz), 6.7 (1H, d, J = 7.6 Hz), 2.34 (3H, s). Anal. ($C_{16}H_{13}F_3N_2O_3 \cdot {}^1/_6H_2O$) C, H, N.

4-Fluoro-2-[3-(3-trifluoromethylphenyl)ureido]-benzoic acid (2f) was prepared according to general procedure A. The product was recrystallized (EtOH−0.5 M HCl). Yield: 53%. Mp: 176−179 °C. ^{1}H NMR (DMSO-d_6): δ 13.62 (1H, br s), 10.76 (1H, s), 10.33 (1H, s), 8.27 (1H, d, J = 12 Hz), 8.05 (1H, dd, J = 12 Hz, 8 Hz), 8.01 (1H, s), 7.72 (1H, d, J = 8.3 Hz), 7.53 (1H, t, J = 8.3 Hz), 7.34 (1H, d, J = 8.3 Hz), 6.9 (1H, t, J = 8 Hz). Anal. ($C_{15}H_{10}F_4N_2O_3$) C, H, N.

4-Chloro-2-[3-(3-trifluoromethylphenyl)ureido]-benzoic acid (2g) was prepared according to general procedure A. The product was recrystallized (acetone−0.5 M HCl). Yield: 63%. Mp: 167−168 °C. ^{1}H NMR (DMSO-d_6): δ 13.78 (1H, br s), 10.69 (1H, s), 10.32 (1H, s), 8.53 (1H, s), 8.01 (1H, s), 7.98 (1H, d, J = 8.0 Hz), 7.71 (1H, d, J = 7.8 Hz), 7.53 (1H, t, J = 7.8 Hz), 7.35 (1H, d, J = 7.8 Hz), 7.13 (1H, d, J = 8.0 Hz). Anal. ($C_{15}H_{10}ClF_3N_2O_3$) C, H, N.

4-Bromo-2-[3-(3-trifluoromethylphenyl)ureido]-benzoic acid (2h) was prepared according to general procedure A. The product was recrystallized (acetone−0.5 M HCl). Yield: 58%. Mp: 177−178 °C. ^{1}H NMR (DMSO-d_6): δ 13.79 (1H, br s), 10.61 (1H, s), 10.31 (1H, s), 8.68 (1H, s), 8.01 (1H, s), 7.88 (1H, d, J = 8.3 Hz), 7.70 (1H, d, J = 8.1 Hz), 7.53 (1H, t, J = 8.1 Hz), 7.34 (1H, d, J = 8.1 Hz), 7.26 (1H, d, J = 8.3 Hz). Anal. ($C_{15}H_{10}BrF_3N_2O_3$) C, H, N.

4-Trifluoromethyl-2-[3-(3-trifluoromethylphenyl)ureido]benzoic Acid (2i). 2-Nitro-4-trifluoromethylbenzoic acid (**10**) (0.96 g, 4.1 mmol) was dissolved in EtOH (40 mL, 96%) and Pd/C (5%, 50 mg) was added. The mixture was hydrogenated by stirring at 1 atm for 1 h, filtered through Celite, and evaporated to dryness under reduced pressure. The product (**12i**) was suspended in toluene and 3-trifluoromethylphenyl isocyanate (**11b**) (1.09 g, 5.8 mmol) was added. The mixture was stirred at room temperature for 24 h, the reaction mixture was cooled, and the product was filtered off and washed with toluene and heptane to yield **2i** as a colorless crystalline solid (1.37 g, 87%). Mp: 178−179 °C. ^{1}H NMR (DMSO-d_6): δ 14.07 (1H, br s), 10.66 (1H, s), 10.37 (1H, s), 8.90 (1H, s), 8.16 (1H, d, J = 8.3 Hz), 8.01 (1H, s), 7.73 (1H, d, J = 7.9 Hz), 7.54 (1H, t, J = 7.9 Hz), 7.4 (1H, d, J = 8.3 Hz), 7.35 (1H, d, J = 7.9 Hz). Anal. ($C_{16}H_{10}F_6N_2O_3$) C, H, N.

4-Nitro-2-[3-(3-trifluoromethylphenyl)ureido]-benzoic acid (2j) was prepared according to general procedure A. The product was recrystallized (EtOH−0.5 M HCl). Yield: 56%. Mp: 206−207 °C. ^{1}H NMR (DMSO-d_6): δ 14.28 (1H, br s), 10.67 (1H, s), 10.41 (1H, s), 9.32 (1H, s), 8.19 (1H, d, J = 8.8 Hz), 8.01 (1H, s), 7.84 (1H, d, J = 8.8 Hz), 7.73 (1H, d, J = 8.0 Hz), 7.54 (1H, t, J = 8.0 Hz), 7.36 (1H, d, J = 8.0 Hz). Anal. ($C_{15}H_{10}F_3N_2O_5$) C, H, N.

3-[3-(3-Trifluoromethylphenyl)ureido]naphthalene-2-carboxylic acid (3) was prepared according to general procedure A. The crude product was dissolved in 0.5 M NaOH and precipitated by addition of 4 M HCl. Yield: 73%. Mp: 197−198 °C. ^{1}H NMR (DMSO-d_6): δ 13.75 (1H, br s), 10.45 (1H, s), 10.20 (1H, s), 8.81 (1H, s), 8.68 (1H, s), 8.10 (1H, s), 8.00 (1H, d, J = 8.2 Hz), 7.86 (1H, d, J = 8.2 Hz), 7.72 (1H, d, J = 7.3 Hz), 7.59 (1H, t, J = 7.3 Hz), 7.53 (1H, t, J = 8.2 Hz), 7.44 (1H, t, J = 8.2 Hz), 7.33 (1H, d, J = 7.3 Hz). Anal. ($C_{19}H_{13}F_3N_2O_3 \cdot {}^1/_4H_2O$) C, H, N.

4-Chloro-2-(3-phenylureido)benzoic acid (4) was prepared according to general procedure A. The product was recrystallized (acetone−0.5 M HCl). Yield: 78%. Mp: 186−188 °C. ^{1}H NMR (DMSO-d_6): δ 13.67 (1H, s), 10.49 (1H, s), 9.94 (1H, s), 8.53 (1H, s), 7.95 (1H, d, J = 8.5 Hz), 7.52 (2H, d, J = 8 Hz), 7.29 (2H, t, J = 8 Hz), 7.09 (1H, d, J = 8.5 Hz), 7.00 (1H, t, J = 8 Hz). Anal. ($C_{14}H_{11}ClN_2O_3$) C, H, N.

4-Chloro-2-[3-(2-fluorophenyl)ureido]benzoic acid (5a) was prepared according to general procedure A. The product was recrystallized (EtOH−0.5 M HCl). Yield: 46%. Mp: 195−196 °C. ^{1}H NMR (DMSO-d_6): δ 13.63 (1H, s), 10.46 (1H, s), 9.77 (1H, s), 8.44 (1H, s), 7.94 (1H, d, J = 8.5), 7.83 (1H, t, J = 7.8 Hz), 7.2 (4H, m). Anal. ($C_{14}H_{10}ClFN_2O_3$) C, H, N.

4-Chloro-2-[3-(2-chlorophenyl)ureido]benzoic acid (5b) was prepared according to general procedure A. The product was recrystallized (EtOH−0.5 M HCl). Yield: 32%. Mp: 181−183 °C. ^{1}H NMR (DMSO-d_6): δ 13.61 (1H, br s), 10.50 (1H, s), 9.53 (1H, s), 8.44 (1H, s), 7.93 (1H, d, J = 8.1 Hz), 7.79 (1H, d, J = 8.5 Hz), 7.49 (1H, d, J = 8.1 Hz), 7.33 (1H, t, J = 8.1 Hz), 7.19 (1H, t, J = 8.1 Hz), 7.11 (1H, d, J = 8.5 Hz). Anal. ($C_{14}H_{10}Cl_2N_2O_3$) C, H, N.

2-[3-(2-Bromophenyl)ureido]-4-chlorobenzoic acid (5c) was prepared according to general procedure A. The product was recrystallized (acetone−H_2O). Yield: 48%. Mp: 195−197 °C. ^{1}H NMR (DMSO-d_6): δ 13.63 (1H, br s), 10.52 (1H, s), 9.46 (1H, s), 8.48 (1H, s), 7.93 (1H, d, J = 8.6 Hz), 7.66 (1H, d, J = 8 Hz), 7.60 (1H, d, J = 8 Hz), 7.38 (1H, t, J = 8 Hz), 7.14 (1H, t, J = 8 Hz), 7.09 (1H, d, J = 8.6 Hz). Anal. ($C_{14}H_{10}BrClN_2O_3$) C, H, N.

4-Chloro-2-[3-(2-iodophenyl)ureido]benzoic acid (5d) was prepared according to general procedure B and the product recrystallized (EtOAc−heptane). Yield: 36%. Mp: 198−201 °C. ^{1}H NMR (DMSO-d_6): δ 13.64 (1H, br s), 10.54 (1H, br s), 9.37 (1H, s), 8.53 (1H, s), 7.93 (1H, d, J = 8.6 Hz), 7.89 (1H, d, J = 7.5 Hz), 7.44 (1H, d, J = 7.5 Hz), 7.40 (1H, t, J = 7.5 Hz), 7.09 (1H, d, J = 8.6 Hz), 7.00 (1H, t, J = 7.5 Hz). Anal. ($C_{14}H_{10}ClIN_2O_3$) C, H, N.

4-Chloro-2-[3-(2-trifluoromethylphenyl)ureido]-benzoic acid (5e) was prepared according to general procedure A. The product was recrystallized (EtOAc−heptane). Yield: 62%. Mp: 192−194 °C. ^{1}H NMR (DMSO-d_6): δ 13.70 (1H, s), 10.61 (1H, s), 10.29 (1H, s), 8.54 (1H, s), 8.01 (1H, s), 7.97 (1H, d, J = 8.6 Hz), 7.71 (1H, d, J = 8.3 Hz), 7.53 (1H, dd, J = 8.3, 7.8 Hz), 7.35 (1H, d, J = 7.8 Hz), 7.13 (1H, d, J = 8.6 Hz). Anal. ($C_{15}H_{10}ClF_3N_2O_3$) C, H, N.

2-(3-Biphenyl-2-ylureido)-4-chlorobenzoic acid (5f) was prepared according to general procedure A. The product was recrystallized (EtOAc−heptane). Yield: 65%. Mp: 208−209 °C. ^{1}H NMR (DMSO-d_6): δ 13.66 (1H, br s), 10.58 (1H, s), 10.07 (1H, s), 8.54 (1H, s), 7.97 (1H, d, J = 8.5 Hz), 7.64 (6H, m), 7.44 (2H, t, J = 7.5 Hz), 7.31 (1H, t, J = 7.5 Hz), 7.11 (1H, d, J = 8.5 Hz). Anal. ($C_{20}H_{15}ClN_2O_3 \cdot {}^1/_3H_2O$) C, H, N.

4-Chloro-2-[3-(2-methoxyphenyl)ureido]benzoic acid (5g) was prepared according to general procedure A. The product was recrystallized (EtOH−0.5 M HCl). Yield: 67%. Mp: 181−183 °C. ^{1}H NMR (DMSO-d_6): δ 13.61 (1H, br s),

10.39 (1H, s), 9.16 (1H, s), 8.38 (1H, s), 7.90 (1H, d, J = 8.6 Hz), 7.76 (1H, d, J = 7.5 Hz), 7.06 (3H, m), 6.90 (1H, t, J = 7.5 Hz). Anal. ($C_{15}H_{13}ClN_2O_4$) C, H, N.

4-Chloro-2-[3-(2-hydroxyphenyl)ureido]benzoic acid (5h) was prepared according to general procedure C and the product recrystallized (acetone−H_2O). Yield: 58%. Mp: 179−180 °C. ^{1}H NMR (DMSO-d_6): δ 13.42 (1H, br s), 10.31 (1H, s), 9.69 (1H, s), 9.13 (1H, s), 8.41 (1H, s), 7.90 (1H, d, J = 8.8 Hz), 7.66 (1H, d, J = 7.9 Hz), 7.08 (1H, d, J = 8.8 Hz), 6.89 (2H, m), 6.76 (1H, t, J = 7.9 Hz). Anal. ($C_{14}H_{11}ClN_2O_4 \cdot {}^1/_3H_2O$) C, H, N.

4-Chloro-2-[3-(3-fluorophenyl)ureido]benzoic acid (6a) was prepared according to general procedure A. The product was recrystallized (EtOH−0.5 M HCl). Yield: 85%. Mp: 175−177 °C. ^{1}H NMR (DMSO-d_6): δ 13.74 (1H, br s), 10.57 (1H, s), 10.17 (1H, s), 8.51 (1H, s), 7.96 (1H, d, J = 8.5 Hz), 7.51 (1H, d, J = 12 Hz), 7.32 (1H, dd, J = 12 Hz, 8.2 Hz), 7.22 (1H, d, J = 8.2 Hz), 7.11 (1H, d, J = 8.5 Hz), 6.81 (1H, t, J = 8.2 Hz). Anal. ($C_{14}H_{10}ClFN_2O_3 \cdot {}^1/_3H_2O$) C, H, N.

4-Chloro-2-[3-(3-chlorophenyl)ureido]benzoic acid (6b) was prepared according to general procedure A. The product was recrystallized (EtOAc−heptane). Yield: 78%. Mp: 196−198 °C. ^{1}H NMR (DMSO-d_6): δ 10.47 (1H, s), 10.07 (1H, s), 8.43 (1H, s), 7.87 (1H, d, J = 8.6 Hz), 7.65 (1H, s), 7.25 (2H, m), 7.03 (1H, d, J = 8.6 Hz), 6.96 (1H, d, J = 7.6 Hz). Anal. ($C_{14}H_{10}Cl_2N_2O_3$) C, H, N.

2-[3-(3-Bromophenyl)ureido]-4-chlorobenzoic acid (6c) was prepared according to general procedure A. The crude product was purified by column chromatography (EtOAc−MeOH). Yield: 61%. Mp: 210−213 °C. ^{1}H NMR (DMSO-d_6): δ 13.63 (1H, br s), 10.50 (1H, s), 9.45 (1H, s), 8.47 (1H, s), 7.93 (1H, d, J = 8.8 Hz), 7.66 (1H, d, J = 7.9 Hz), 7.60 (1H, d, J = 7.9 Hz), 7.37 (1H, t, J = 7.9 Hz), 7.14 (1H, d, J = 7.9 Hz), 7.10 (1H, d, J = 8.8 Hz). Anal. ($C_{14}H_{10}BrClN_2O_3$) C, H, N.

4-Chloro-2-[3-(3-iodophenyl)ureido]benzoic acid (6d) was prepared according to general procedure A. The product was recrystallized (EtOAc−heptane). Yield: 37%. Mp: 192−193 °C. ^{1}H NMR (DMSO-d_6): δ 13.80 (1H, br s), 10.70 (1H, s), 10.07 (1H, s), 8.50 (1H, s), 8.04 (1H, s), 7.96 (1H, d, J = 8.6 Hz), 7.45 (1H, d, J = 7.7 Hz), 7.35 (1H, d, J = 7.7 Hz), 7.10 (2H, m). Anal. ($C_{14}H_{10}ClIN_2O_3$) C, H, N.

2-(3-Biphenyl-3-ylureido)-4-chlorobenzoic Acid (6f). 3-Iodobenzoic acid (**11m**) (4.96 g, 20 mmol), phenyl boronic acid (2.68 g, 22 mmol), Na_2CO_3 (6.36 g, 60 mmol), and $Pd(OAc)_2$ (44 mg, 0.14 mmol) were dissolved in water (80 mL) and heated at 50 °C for 1 h. The mixture was filtered, washed with EtOAc, acidified with HCl, and extracted into EtOAc, dried with Na_2SO_4, evaporated, and recrystallized (EtOAc−heptane) to yield 3-phenylbenzoic acid (**9b**) (3.6 g, 91%) as a colorless crystalline solid. **6f** was then synthesized from **9b** and **12g** as described in general method B and recrystallized (EtOAc−heptane). Yield: 46%. Mp: 181−183 °C. ^{1}H NMR (DMSO-d_6): δ 13.70 (1H, br s), 10.58 (1H, s), 10.08 (1H, s), 8.56 (1H, s), 7.97 (1H, d, J = 8.6 Hz), 7.86 (1H, s), 7.63 (2H, d, J = 7.5 Hz), 7.50 (3H, m), 7.40 (1H, d, J = 7.5 Hz), 7.37 (1H, d, J = 7.5 Hz), 7.30 (1H, d, J = 7.5 Hz), 7.11 (1H, d, J = 8.6 Hz). Anal. ($C_{20}H_{15}ClN_2O_3$) C, H, N.

4-Chloro-2-[3-(3-methoxyphenyl)ureido]benzoic acid (6g) was prepared according to general procedure A. The product was recrystallized (EtOAc−heptane). Yield: 88%. Mp: 166−169 °C. ^{1}H NMR (DMSO-d_6): δ 13.70 (1H, s), 10.51 (1H, s), 9.95 (1H, s), 8.52 (1H, s), 7.95 (1H, d, J = 8.6 Hz), 7.18 (2H, m), 7.18 (1H, d, J = 8.6 Hz), 7.05 (1H, d, J = 8.2 Hz), 6.58 (1H, d, J = 8.2 Hz), 3.73 (3H, s). Anal. ($C_{15}H_{13}ClN_2O_4$) C, H, N.

4-Chloro-2-[3-(3-hydroxyphenyl)ureido]benzoic acid (6h) was prepared according to general procedure C and the product recrystallized (acetone−H_2O). Yield: 58%. Mp: 160−162 °C. ^{1}H NMR (DMSO-d_6): δ 13.63 (1H, s), 10.43 (1H, s), 9.82 (1H, s), 9.34 (1H, s), 8.50 (1H, s), 7.95 (1H, d, J = 8.6 Hz), 7.07 (3H, m), 6.88 (1H, d, J = 8.0 Hz), 6.40 (1H, d, J = 8.0 Hz). Anal. ($C_{14}H_{11}ClN_2O_4 \cdot {}^1/_3H_2O$) C, H, N.

4-Chloro-2-[3-(4-fluorophenyl)ureido]benzoic acid (7a) was prepared according to general procedure A. The product was recrystallized (EtOAc−heptane). Yield: 43%. Mp: 194−195 °C. ^{1}H NMR (DMSO-d_6): δ 13.56 (1H, br s), 10.51 (1H, s), 9.97 (1H, s), 8.52 (1H, s), 7.95 (1H, d, J = 8.5 Hz), 7.52 (2H, m), 7.12 (3H, m). Anal. ($C_{14}H_{10}ClFN_2O_3$) C, H, N.

4-Chloro-2-[3-(4-chlorophenyl)ureido]benzoic acid (7b) was prepared according to general procedure A. The product was recrystallized (EtOAc−heptane). Yield: 44%. Mp: 205−207 °C. ^{1}H NMR (DMSO-d_6): δ 13.67 (1H, br s), 10.55 (1H, s), 10.08 (1H, s), 8.51 (1H, s), 7.96 (1H, d, J = 8.6 Hz), 7.54 (2H, d, J = 8.9 Hz), 7.34 (2H, d, J = 8.9 Hz), 7.10 (1H, d, J = 8.6 Hz). Anal. ($C_{14}H_{10}Cl_2N_2O_3$) C, H, N.

2-[3-(4-Bromophenyl)ureido]-4-chlorobenzoic acid (7c) was prepared according to general procedure A. The product was recrystallized (EtOH−0.5 M HCl). Yield: 31%. Mp: 209−209 °C. ^{1}H NMR (DMSO-d_6): δ 13.71 (1H, br s), 10.55 (1H, s), 10.10 (1H, s), 8.51 (1H, s), 7.96 (1H, d, J = 8.5 Hz), 7.50 (2H, d, J = 7.7 Hz), 7.46 (2H, d, J = 7.7 Hz), 7.1 (1H, d, J = 8.5 Hz). Anal. ($C_{14}H_{10}BrClFN_2O_3 \cdot {}^1/_4H_2O$) C, H, N.

4-Chloro-2-[3-(4-iodophenyl)ureido]benzoic acid (7d) was prepared according to general procedure A. The product was recrystallized (acetone−H_2O). Yield: 38%. Mp: 209−210 °C. ^{1}H NMR (DMSO-d_6): δ 10.43 (1H, s), 9.96 (1H, s), 8.40 (1H, s), 7.85 (1H, d, J = 8.5 Hz), 7.51 (2H, d, J = 8.5 Hz), 7.26 (2H, d, J = 8.5 Hz), 7.00 (1H, d, J = 8.5 Hz). Anal. ($C_{14}H_{10}ClIN_2O_3$) C, H, N.

4-Chloro-2-[3-(4-trifluoromethylphenyl)ureido]-benzoic acid (7e) was prepared according to general procedure A. The crude product was purified by column chromatography (EtOAc−MeOH). Yield: 56%. Mp: 167−169 °C. ^{1}H NMR (DMSO-d_6): δ 10.61 (1H, s), 10.37 (1H, s), 8.51 (1H, s), 7.97 (1H, d, J = 8.6 Hz), 7.73 (2H, d, J = 8.9 Hz), 7.65 (2H, d, J = 8.9), 7.13 (1H, d, J = 8.6 Hz). Anal. ($C_{15}H_{10}ClF_3N_2O_3$) C, H, N.

2-(3-Biphenyl-4-ylureido)-4-chlorobenzoic acid (7f) was prepared according to general procedure A. The product was recrystallized (EtOAc−heptane). Yield: 86%. Mp: 193−194 °C. ^{1}H NMR (DMSO-d_6): δ 13.55 (1H, br s), 10.29 (1H, s), 9.13 (1H, s), 8.43 (1H, s), 7.88 (1H, d, J = 8.6 Hz), 7.40 (9H, m), 7.03 (1H, d, J = 8.6 Hz). Anal. ($C_{20}H_{15}ClN_2O_3$) C, H, N.

4-Chloro-2-[3-(4-methoxyphenyl)ureido]benzoic acid (7g) was prepared according to general procedure A. The product was recrystallized (EtOAc−heptane). Yield: 73%. Mp: 194−195 °C. ^{1}H NMR (DMSO-d_6): δ 10.45 (1H, s), 9.72 (1H, s), 8.54 (1H, s), 7.94 (1H, d, J = 8.6 Hz), 7.40 (2H, d, J = 8.9 Hz), 7.07 (1H, d, J = 8.6 Hz), 6.88 (2H, d, J = 8.9 Hz), 3.72 (3H, s). Anal. ($C_{15}H_{13}ClN_2O_4$) C, H, N.

4-Chloro-2-[3-(4-hydroxyphenyl)ureido]benzoic acid (7h) was prepared according to general procedure C and the product recrystallized (acetone−H_2O). Yield: 76%. Mp: 212−214 °C. ^{1}H NMR (DMSO-d_6): δ 13.63 (1H, br s), 10.43 (1H, s), 9.55 (1H, s), 9.15 (1H, s), 8.54 (1H, s), 7.93 (1H, d, J = 8.6 Hz), 7.25 (2H, d, J = 8.8 Hz), 7.06 (1H, d, J = 8.6 Hz), 6.69 (2H, d, J = 8.8 Hz). Anal. ($C_{14}H_{11}ClN_2O_4$) C, H, N.

4-Chloro-2-(3-cyclohexylureido)benzoic Acid (8a). 2-Amino-4-chlorobenzoic acid (**12g**) (340 mg, 2.0 mmol) was dissolved in aqueous $KHCO_3$ (2 mL, 10%) together with cyclohexyl isocyanate (**14a**) (0.27 mL, 2.1 mmol) and the mixture was stirred overnight. The mixture was then acidified with concentrated HCl and the resulting precipitate was filtered off and washed with water. Recrystallization from hot EtOH with water gave **8a** as a colorless crystalline solid (430 mg, 72%). Mp: 179−181 °C. ^{1}H NMR (DMSO-d_6): δ 13.51 (1H, br s), 10.13 (1H, s), 7.54 (1H, br s), 8.54 (1H, s), 7.89 (1H, d, J = 8.5 Hz), 6.99 (1H, d, J = 8.6 Hz), 3.39 (1H, br s), 1.70 (5H, m), 1.20 (5H, m). Anal. ($C_{14}H_{17}ClN_2O_3 \cdot {}^1/_6H_2O$) C, H, N.

4-Chloro-2-(3-pyridin-2-ylureido)benzoic acid (8b) was prepared according to general procedure B but was not extracted into water but precipitated directly from EtOAc with heptane and was recrystallized (EtOAc−heptane). Yield: 73%. Mp: 222−224 °C. ^{1}H NMR (DMSO-d_6): δ 13.45 (1H, s), 10.96 (1H, br s), 9.97 (1H, s), 8.27 (1H, s), 8.24 (1H, d, J = 5 Hz), 7.93 (1H, d, J = 8.5 Hz), 7.83 (1H, d, J = 8 Hz), 7.69 (1H, t, J = 8 Hz), 6.95 (1H, dd, J = 8 Hz, 5 Hz), 6.88 (1H, d, J = 8.5 Hz). Anal. ($C_{13}H_{10}ClN_2O_3 \cdot H_2O$) C, H, N.

4-Chloro-2-(3-pyridin-3-ylureido)benzoic acid (8c) was prepared according to general procedure B but was not extracted into water but precipitated directly from EtOAc with heptane and was recrystallized (DMF). Yield: 26%. Mp: >250 °C. ^{1}H NMR (DMSO-d_6): δ 10.65 (1H, s), 10.17 (1H, s), 8.67 (1H, s), 8.53 (1H, s), 8.22 (1H, d, J = 4.5 Hz), 7.97 (2H, m), 7.34 (1H, dd, J = 8.5 Hz, 4.5 Hz), 7.12 (1H, d, J = 8.5 Hz). Anal. ($C_{13}H_{10}ClN_3O_3 \cdot H_2O$) C, H, N.

4-Chloro-2-(3-pyridin-4-ylureido)benzoic acid (8d) was prepared according to general procedure B but was not extracted into water but precipitated directly from EtOAc with heptane and was recrystallized (DMF). Yield: 10%. Mp: >250 °C. ^{1}H NMR (DMSO-d_6): δ 11.23 (1H, s), 10.57 (1H, s), 8.48 (1H, s), 8.41 (2H, d, J = 5.3 Hz), 7.98 (1H, d, J = 8.5 Hz), 7.58 (2H, d, J = 5.3 Hz), 7.13 (1H, d, J = 8.5 Hz). Anal. ($C_{13}H_{10}ClN_3O_3 \cdot {}^3/_2H_2O$) C, H, N.

4-Chloro-2-(3-thiophen-2-ylureido)benzoic acid (8e) was prepared according to general procedure B and the product recrystallized (EtOAc−heptane). Yield: 37%. Mp: 199−200 °C. ^{1}H NMR (DMSO-d_6): δ 13.76 (1H, br s), 11.04 (1H, s), 10.75 (1H, s), 8.55 (1H, s), 7.96 (1H, d, J = 8.6 Hz), 7.11 (1H, d, J = 8.6 Hz), 6.93 (1H, d, J = 5.5 Hz), 6.83 (1H, dd, J = 5.5 Hz, 4 Hz), 6.60 (1H, d, J = 4 Hz). Anal. ($C_{12}H_9ClN_2O_3$) C, H, N.

4-Chloro-2-(3-thiophen-3-ylureido)benzoic acid (8f) was prepared according to general procedure B and the product recrystallized (EtOAc−heptane). Yield: 26%. Mp: >250 °C. ^{1}H NMR (DMSO-d_6): δ 13.69 (1H, br s), 10.56 (1H, s), 10.29 (1H, s), 8.58 (1H, s), 7.95 (1H, d, J = 8.9 Hz), 7.45 (1H, m), 7.35 (1H, m), 7.08 (2H, m). Anal. ($C_{12}H_9ClN_2O_3$) H, N, C: calcd 48.57; found 49.19.

4-Chloro-2-(3-naphthalen-1-ylureido)benzoic acid (8g) was prepared according to general procedure A. The product was recrystallized (EtOH−0.5 M HCl). Yield: 62%. Mp: 206−209 °C. ^{1}H NMR (DMSO-d_6): δ 13.60 (1H, s), 10.60 (1H, s), 9.80 (1H, s), 8.55 (1H, s), 8.10 (1H, d, J = 8.0 Hz), 7.94 (2H, m), 7.77 (1H, d, J = 8.0 Hz), 7.67 (1H, d, J = 7.4 Hz), 7.53 (3H, m), 7.09 (1H, d, J = 8.6). Anal. ($C_{18}H_{13}ClN_2O_3$) C, H, N.

4-Chloro-2-(3-naphthalen-2-ylureido)benzoic acid (8h) was prepared according to general procedure B and the product recrystallized (EtOAc−heptane). Yield: 46%. Mp: 176−179 °C (decomp). ^{1}H NMR (DMSO-d_6): δ 10.60 (1H, s), 10.19 (1H, s), 8.59 (1H, s), 8.19 (1H, s), 7.97 (d, 1H, J = 8.2 Hz), 7.84 (4H, m), 7.45 (3H, m), 7.12 (1H, d, J = 8.2 Hz). Anal. ($C_{18}H_{13}ClN_2O_3$) C, H, N.

2-(3-Anthracen-2-ylureido)-4-chlorobenzoic acid (8i) was prepared according to general procedure B and the product recrystallized (EtOAc−heptane). Yield: 69%. Mp: 230 °C (dec). ^{1}H NMR (DMSO-d_6): δ 10.63 (1H, s), 10.25 (1H, s), 8.60 (1H, s), 8.48 (1H, s), 8.42 (1H, s), 8.35 (1H, s), 8.04 (3H, m), 7.98 (1H, d, J = 8.6 Hz), 7.56 (1H, d, J = 9.0 Hz), 7.46 (2H, m), 7.13 (1H, d, J = 9.0 Hz). Anal. ($C_{22}H_{15}ClN_2O_3$) C, H, N.

2-(3-Benzo[1,3]dioxol-5-ylureido)-4-chlorobenzoic acid (8j) was prepared according to general procedure A. The product was recrystallized (acetone−H_2O). Yield: 64%. Mp: 183−185 °C. ^{1}H NMR (DMSO-d_6): δ 13.67 (1H, br s), 10.48 (1H, s), 9.80 (1H, s), 8.51 (1H, s), 7.94 (1H, d, J = 8.6 Hz), 7.20 (1H, s), 7.08 (1H, d, J = 8.6 Hz), 6.86 (2H, m), 5.98 (2H, s) Anal. ($C_{15}H_{11}ClN_2O_3$) C, H, N.

4-Chloro-2-[3-(1*H*-indol-2-yl)ureido]benzoic acid (8k) was prepared according to general procedure B but was not extracted into water but precipitated directly and was filtered and washed with Et_2O and EtOH. Yield: 21%. Mp: >250 °C. ^{1}H NMR (DMSO-d_6): δ 13.75 (1H, br s), 10.98 (1H, s), 10.67 (1H, s), 10.58 (1H, s), 8.61 (1H, s), 7.97 (1H, d, J = 8.5 Hz), 7.36 (2H, m), 7.13 (1H, d, J = 8.5 Hz), 6.93 (2H, m), 5.98 (1H, s). Anal. ($C_{16}H_{12}ClN_2O_3$) C, H, N.

Stable Cell Lines. HEK293 cell lines stably expressing homomeric human GluR5−1a and human GluR6 were established as described previously.[14] 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,[15] 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, 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.[14,16] Cells were cultured to 80−90% confluency before plating. The cells were rinsed with 10 mL of PBS, then 1.5 mL of trypsin−EDTA [0.1% (w/v) trypsin] was added, and the mixture 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) 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 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 either 2 μM domoate (for GluR5), 0.2 μM domoate (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 ethanol or DMSO, and final concentration never exceeding 1.0%.

Ligand Binding Studies. GluR5-expressing cells were harvested and 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 27 000*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.[14] Briefly, binding to GluR5 receptors was performed using 3 nM [^{3}H]ATPA and 46−84 μg 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.

Patch-Clamp Electrophysiology. All experiments were performed in voltage clamp using conventional whole cell patch clamp methods, as described previously.[14] The following salt solutions were used. Extracellular solution (mM): NaCl (140), KCl (4), $CaCl_2$ (2), $MgCl_2$ (4), HEPES (10, pH =7.4). Intracellular solution (mM): KCl (120), KOH (31), $MgCl_2$ (1.8), EGTA (10), HEPES (10, pH = 7.2).

Prior to each experiment, HEK293 cells stably expressing GluR5 receptors were incubated for 10−20 min in extracellulary solution containing concanavaline A (0.25 mg/mL) to remove rapid desensitization. During experiments, cells were held at a holding potential of −60 mV and every 45 s subjected to a 1 s pulse of 1 mM Glu, delivered to the recording chamber through a custom-made gravity-driven flow pipe. After obtaining responses of stable amplitude, the extracellular solution as well as the Glu-containing solution were switched to solutions containing compound **6c** at the desired concentration. The compound was present until responses of a new stable amplitude were achieved. Currents were measured at the peak of the responses, and the effect of compounds was calculated as the current at compound equilibrium divided by the current

evoked by the pulse immediately before the compound was included. The concentration−response curve was obtained by fitting data (means ± SEM) to the equation $y = 100 - (ax^n/b + x^n)$, where a = maximal response ("100"), $b = (IC_{50})^n$, and n is the Hill coefficient. The patch clamp experiments were performed at room temperature (20−22 °C).

ATPA-Induced Rigidity. Rigidity in mice was induced by intravenous administration of ATPA. ATPA is systematically active[17] and has agonistic activity primarily at the GluR5 subtype.[18,19] 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.[20,21] ATPA was dissolved in H_2O by addition of a 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 every 5 min for a total period of 30 min after ATPA administration. The data are presented as percent of mice for each experimental group showing rigidity as a function of time.

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References

(1) Bräuner-Osborne, H.; Egebjerg, J.; Nielsen, E. Ø.; Madsen, U.; Krogsgaard-Larsen, P. Ligands for glutamate receptors: Design and therapeutic prospects. *J. Med. Chem.* **2000**, *43*, 2609−2645.

(2) Dingledine, R.; Borges, K.; Bowie, D.; Traynelis, S. F. The glutamate receptor ion channels. *Pharmacol. Rev.* **1999**, *51*, 7−61.

(3) Lee, G. J. Pharmacology of AMPA/kainate receptor ligands and their therapeutic potential in neurological and psychiatric disorders. *Drugs* **2000**, *59*, 33−78.

(4) Parsons, C. G.; Danysz, W.; Quack, G. Glutamate in CNS disorders as a target for drug development: An update. *Drug News Perspect.* **1998**, *11*, 523−569.

(5) O'Neill, M. J.; Bond, A.; Ornstein, P. L.; Ward, M. A.; Hicks, C. A.; Hoo, K.; Bleakman, D.; Lodge, D. Decahydroisoquinolines: Novel competitive AMPA/kainate antagonists with neuroprotective effects in global cerebral ischaemia. *Neuropharmacology* **1998**, *37*, 1211−1222.

(6) O'Neill, M. J.; Bogaert, L.; Hicks, C. A.; Bond, A.; Ward, M. A.; Ebinger, G.; Ornstein, P. L.; Michotte, Y.; Lodge, D. LY377770, a novel iGlu5 kainate receptor antagonist with neuroprotective effects in global and focal cerebral ischaemia. *Neuropharmacology* **2000**, *39*, 1575−1588.

(7) Simmons, R. M.; Li, D. L.; Hoo, K. H.; Deverill, M.; Ornstein, P. L.; Iyengar, S. Kainate GluR5 receptor subtype mediates the nociceptive response to formalin in the rat. *Neuropharmacology* **1998**, *37*, 25−36.

(8) Smolders, D.; De Foer, B.; Pouillon, M.; Casselman, J.; De Schepper, A. M. Idiopathic hypertrophic intracranial pachymeningitis. *JBR-BTR* **2002**, *85*, 154−155.

(9) Filla, S. A.; Winter, M. A.; Johnson, K. W.; Bleakman, D.; Bell, M. G.; Bleisch, T. J.; Castano, A. M.; Clemens-Smith, A.; Del Prado, M.; Dieckman, D. K.; Dominguez, E.; Escribano, A.; Ho, K. H.; Hudziak, K. J.; Katofiasc, M. A.; Martinez-Perez, J. A.; Mateo, A.; Mathes, B. M.; Mattiuz, E. L.; Ogden, A. M. L.; Phebus, L. A.; Stack, D. R.; Stratford, R. E.; Ornstein, P. L. Ethyl (3*S*,4a*R*,6*S*,8a*R*)-6-(4-Ethoxycarbonylimidazol-1-ylmethyl)decahydroisoquinoline-3-carboxylic ester: A prodrug of a GluR5 kainate receptor antagonist active in two animal models of acute migraine. *J. Med. Chem.* **2002**, *45*, 4383−4386.

(10) Bleakman, D.; Schoepp, D. D.; Ballyk, B.; Bufton, H.; Sharpe, E. F. Pharmacological Discrimination of GluR5 and GluR6 Kainate Receptor Subtypes by (3*S*,4a*R*,6*R*,8a*R*)-6-[2-(1(2)*H*-tetrazole-5-yl)ethyl]decahydroisoquinoline-3-carboxylic acid. *Mol. Pharmacol.* **1996**, *49*, 581−585.

(11) Bleakman, D.; Gates, M. R.; Ogden, A. M.; Mackowiak, M. Kainate receptor agonists, antagonists and allosteric modulators. *Curr. Pharm. Des.* **2002**, *8*, 873−885.

(12) Solyom, S.; Tarnawa, I. Noncompetitive AMPA antagonists of 2,3-benzodiazepine type. *Curr. Pharm. Des.* **2002**, *8*, 913−939.

(13) Christensen, J. K.; Varming, T.; Ahring, P. K.; Jørgensen, T. D.; Nielsen, E. Ø. In vitro characterization of NS3763, a noncompetitive antagonist of GLU_{K5} receptors. *J. Pharmacol. Exp. Ther.* Submitted.

(14) Varming, T.; Ahring, P. K.; Sager, T. N.; Johansen, T. H.; Wätjen, F.; Drejer, J. Optimization of isatine oximes as neuroprotective AMPA receptor antagonists: In vitro and in vivo evaluation of SPD 502, in *Excitatory amino acids: Ten years later*; Turski L., Schoepp D. D., Cavalheiro E. A., Eds.; IOS Press: Amsterdam, 2001; pp 193−195.

(15) White, H. S.; Sarup, A.; Bolvig, T.; Kristensen, A. S.; Petersen, G.; Nelson, N.; Pickering, D. S.; Larsson, O. M.; Frølund, B.; Krogsgaard-Larsen, P.; Schousboe, A. Correlation between anticonvulsant activity and inhibitory action on glial gamma-aminobutyric acid uptake of the highly selective mouse gamma-aminobutyric acid transporter 1 inhibitor 3-hydroxy-4-amino-4,5,6,7-tetrahydro-1,2-benzisoxazole and its N-alkylated analogues. *J. Pharmacol. Exp. Ther.* **2002**, *302*, 636−644.

(16) Nielsen, E. Ø.; Varming, T.; Mathiesen, C.; Jensen, L. H.; Møller, A.; Gouliaev, A. H.; Wätjen, F.; Drejer, J. SPD 502: A water-soluble and in vivo long-lasting AMPA antagonist with neuroprotective activity. *J. Pharmacol. Exp. Ther.* **1999**, *289*, 1492−1501.

(17) Turski, L.; Jacobsen, P.; Honore, T.; Stephens, D. N. Relief of experimental spasticity and anxiolytic/anticonvulsant actions of the alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate antagonist 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(F)quinoxaline. *J. Pharmacol. Exp. Ther.* **1992**, *260*, 742−747.

(18) Clarke, V. R.; Ballyk, B. A.; Hoo, K. H.; Mandelzys, A.; Pellizzari, A.; Bath, C. P.; Thomas, J.; Sharpe, E. F.; Davies, C. H.; Ornstein, P. L.; Schoepp, D. D.; Kamboj, R. K.; Collingridge, G. L.; Lodge, D.; Bleakman, D. A hippocampal GluR5 kainate receptor regulating inhibitory synaptic transmission. *Nature* **1997**, *389*, 599−603.

(19) Bunch, L.; Johansen, T. H.; Bräuner-Osborne, H.; Stensbøl, T. B.; Johansen, T. N.; Krogsgaard-Larsen, P.; Madsen, U. Synthesis and receptor binding affinity of new selective GluR5 ligands. *Bioorg. Med. Chem.* **2001**, *9*, 875−879.

(20) Ornstein, P. L.; Arnold, M. B.; Allen, N. K.; Leander, J. D.; Tizzano, J. P.; Lodge, D.; Schoepp, D. D. (3*SR*,4a*RS*,6*SR*,8a*RS*)-6-(1*H*-tetrazol-5-yl)decahydroisoquinoline-3-carboxylic acid, a novel, competitive, systemically active NMDA and AMPA receptor antagonist. *J. Med. Chem.* **1995**, *38*, 4885−4890.

(21) Ornstein, P. L.; Arnold, M. B.; Allen, N. K.; Bleisch, T.; Borromeo, P. S.; Lugar, C. W.; Leander, J. D.; Lodge, D.; Schoepp, D. D. Structure−activity studies of 6-substituted decahydroisoquinoline-3-carboxylic acid AMPA receptor antagonists. 2. Effects of distal acid bioisosteric substitution, absolute stereochemical preferences, and in vivo activity. *J. Med. Chem.* **1996**, *39*, 2232−2244.

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