A New Highly Selective Metabotropic Excitatory Amino Acid Agonist: 2-Amino-4-(3-hydroxy-5-methylisoxazol-4-yl)butyric Acid

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The homologous series of acidic amino acids, ranging from aspartic acid (**1**) to 2-aminosuberic acid (**5**), and the corresponding series of 3-isoxazolol bioisosteres of these amino acids, ranging from (*RS*)-2-amino-2-(3-hydroxy-5-methylisoxazol-4-yl)acetic acid (AMAA, **6**) to (*RS*)-2-amino-6-(3-hydroxy-5-methylisoxazol-4-yl)hexanoic acid (**10**), were tested as ligands for metabotropic excitatory amino acid receptors ($mGlu_{1\alpha}$, $mGlu_2$, $mGlu_{4a}$, and $mGlu_6$). Whereas AMAA (**6**) and (*RS*)-2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid (AMPA, **7**) are potent and highly selective agonists at *N*-methyl-D-aspartic acid (NMDA) and AMPA receptors, respectively, the higher homologue of AMPA (**7**), (*RS*)-2-amino-4-(3-hydroxy-5-methylisoxazol-4-yl)butyric acid (homo-AMPA, **8**), is inactive at ionotropic excitatory amino acid receptors. Homo-AMPA (**8**), which is a 3-isoxazolol bioisostere of 2-aminoadipic acid (**3**), was, however, shown to be a specific and rather potent agonist at $mGlu_6$, approximately 4 times weaker than the nonselective excitatory amino acid receptor agonist (*S*)-glutamic acid. 2-Aminoadipic acid (**3**), which shows a complex excitatory amino acid synaptic pharmacology, was an agonist at $mGlu_6$ as well as $mGlu_2$. AMPA (**7**) and the higher homologue of homo-AMPA (**8**), (*RS*)-2-amino-5-(3-hydroxy-5-methylisoxazol-4-yl)pentanoic acid (**9**), showed relatively weak agonist effects at $mGlu_6$. It is concluded that homo-AMPA (**8**) is likely to be a useful tool for studies of the pharmacology and physiological role of $mGlu_6$. We describe a new versatile synthesis of this homologue of AMPA and the synthesis of compound **10**.

Introduction

(*S*)-Glutamic acid [(*S*)-Glu, (*S*)-**2**] and perhaps also (*S*)-aspartic acid [(*S*)-**1**] are the major excitatory neurotransmitters in the central nervous system and play an important role in many natural processes such as neural plasticity, memory, and neurotoxicity.[1−4] On the basis of pharmacology, electrophysiology, and molecular cloning, the excitatory amino acid (EAA) receptors operated by (*S*)-Glu have been classified into two major classes: the ionotropic glutamic acid (iGlu) receptors and the metabotropic glutamic acid (mGlu) receptors belonging to the superfamily of G protein-coupled receptors.[4] Whereas the former class of EAA receptors comprises the *N*-methyl-D-aspartic acid (NMDA), (*RS*)-2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid (AMPA), and kainic acid subgroups of receptors, all of which are heterogeneous, eight different subtypes of mGlu receptors have, so far, been cloned.[5−11] On the basis of pharmacology, signal transduction pathways, and sequence homology, the mGlu receptors have been subdivided into three groups (I−III). Group I consists of $mGlu_1$ and $mGlu_5$, which are coupled to the hydrolysis of phosphatidylinositol (PI) and are selectively activated by quisqualic acid.[5−7] $mGlu_2$ and $mGlu_3$, which constitute group II, are coupled to inhibition of cyclic AMP formation and are selectively activated by (1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid [(1*S*,3*R*)-ACPD] and (2*S*,1′*R*,2′*R*,3′*R*)-2-(2,3-dicarboxycyclopropyl)glycine (DCG-IV).[8,12,13] Group III comprises $mGlu_4$, $mGlu_6$, $mGlu_7$, and $mGlu_8$, which also are coupled to inhibition of cyclic AMP formation and are selectively activated by (*S*)-2-amino-4-phosphonobutyric acid (L-AP4).[9−11,13]

A prerequisite for the determination of the physiological role and pharmacological importance of the mGlu receptors is the availability of highly selective agonists and antagonists for each subtype of this heterogeneous family of EAA receptors.[14,15] Recently, 2-amino-3-(4-bromo-3-hydroxyisoxazol-5-yl)propionic acid (Br-HIBO), which is a potent AMPA receptor agonist,[16] has been shown to be a mGlu receptor antagonist.[17] Like 1-aminoindan-1,5-dicarboxylic acid,[18] Br-HIBO reduces the function of $mGlu_1$ without affecting significantly other mGlu receptors.[17] However, for the other subtypes of mGlu receptors, no potent and selective antagonists have yet been reported.

We have previously shown that 2-amino-2-(3-hydroxy-5-methylisoxazol-4-yl)acetic acid (AMAA, **6**), a 3-isoxazolol bioisostere of aspartic acid (**1**), is a specific agonist at NMDA receptors,[19] whereas AMPA (**7**), bioisosterically derived from glutamic acid (**2**) (Figure 1), is a highly selective AMPA receptor agonist.[16] Along this line of EAA receptor ligand design, we have synthesized and pharmacologically characterized the two higher homologues of AMPA (**7**), i.e., (*RS*)-2-amino-4-(3-hydroxy-5-methylisoxazol-4-yl)butyric acid (homo-AMPA, **8**)[20] and (*RS*)-2-amino-5-(3-hydroxy-5-methylisoxazol-4-yl)pentanoic acid (**9**).[21] Whereas homo-AMPA (**8**) is completely devoid of effects at the iGlu receptors,[20]

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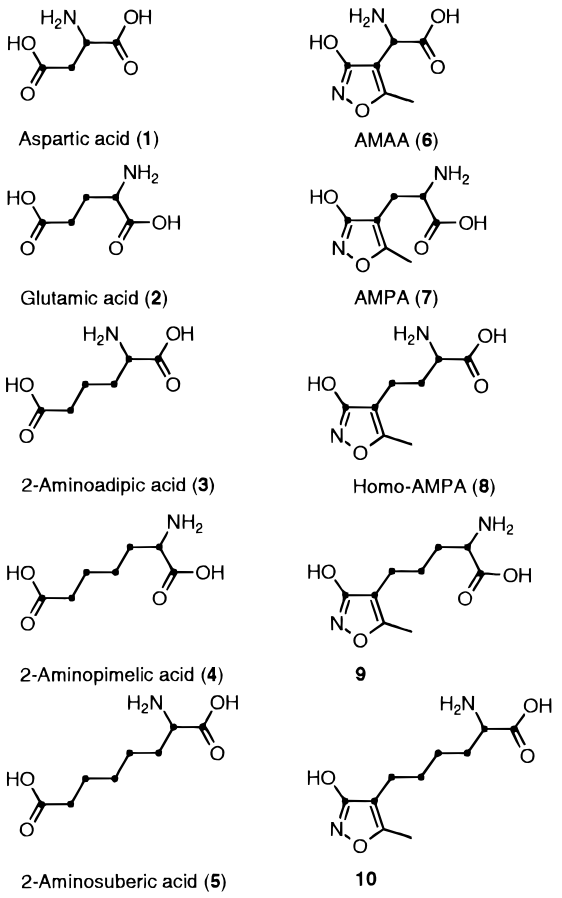


Figure 1. Structures of the acidic amino acids and corresponding 3-isoxazolol bioisosteres tested in this study.

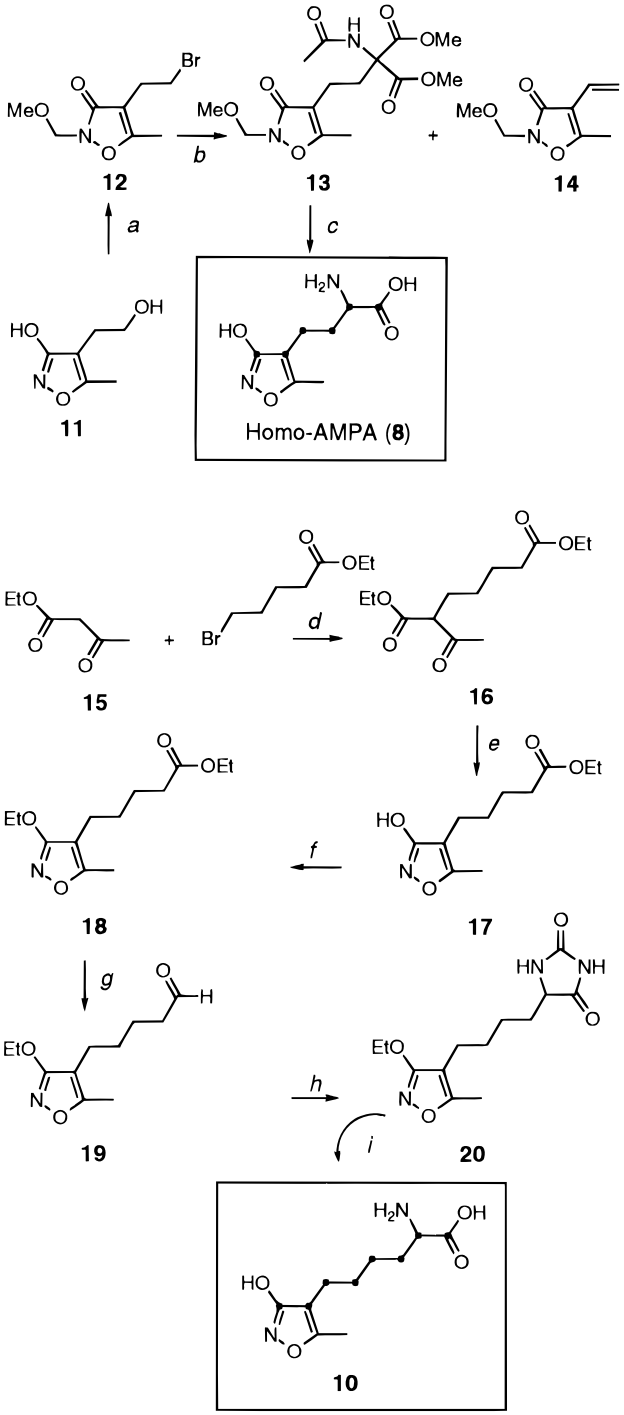
compound **9** was shown to be a very weak AMPA receptor agonist.[21]

We here report the results of a comparative study of the effects of the homologous series of amino acids ranging from aspartic acid (**1**) to 2-aminosuberic acid (**5**) and the corresponding series of 3-isoxazolol amino acids ranging from AMAA (**6**) to (*RS*)-2-amino-6-(3-hydroxy-5-methylisoxazol-4-yl)hexanoic acid (**10**) (Figure 1) on membrane-bound iGlu receptors and representative subtypes of recombinant mGlu receptors. As a notable result of this study, homo-AMPA (**8**) was shown to be a highly selective agonist at $mGlu_6$, 2 times as potent as the *S*-form of its amino acid analogue, 2-aminoadipic acid (**3**) (Figure 1), which was shown also to be an agonist at $mGlu_2$. As part of this study we report the synthesis of compound **10**, which was pharmacologically inactive, and an improved synthesis of homo-AMPA (**8**).

Chemistry

The potential utility of homo-AMPA (**8**) for pharmacological studies of $mGlu_6$ prompted us to develop a new and effective synthesis of this compound (Scheme 1, top) to replace the earlier described low-yield procedure.[20] The key steps in this new reaction sequence are the transformations of 3-hydroxy-4-(2-hydroxyethyl)-5-methylisoxazole (**11**)[22] into compound **12** and subsequently into the precursor for **8**, compound **13**. This reaction was optimized to provide **13** in 62% yield, though with the formation of compound **14** (14%) as an unvoidable side reaction. Compound **13** was easily separated from **14** and transformed into homo-AMPA (**8**).

Scheme 1[a]



[a] Reagents: (a) 1,3,5-trioxane, HBr (62%), then MeOH; (b) $AcNHCH(COOCH_3)_2$, NaH, DMF; (c) CF_3COOH (1 M), IRA-400; (d) NaOEt, NaI; (e) $NH_2OH{\cdot}HCl$, NaOH, then HCl; (f) NaOH, DMF, then EtI; (g) $AlH[CH_2CH(CH_3)_2]_2$ (1 M), toluene; (h) KCN, $(NH_4)_2CO_3$, $MeOH/H_2O$; (i) HCl (6 M).

The synthesis of the new compound, **10**, is also shown in Scheme 1 (bottom). The key steps in this reaction sequence are the regioselective conversion of **16** into the 3-isoxazolol **17** by treatment with the binucleophile hydroxylamine and the reduction of the ester group of **18** into the aldehyde group in **19**. These reactions were optimized to provide respectively **17** in 63% yield and **19** in a yield of 94%. The decomposition of the hydantoin nucleus of **20** to an α-amino acid function and the deprotection of the 3-isoxazolol unit were accomplished in one reaction step to provide **10** in 34% yield.

Table 1. Agonist Activities of the 3-Isoxazolol Amino Acid Bioisosteres at Ionotropic Excitatory Amino Acid Receptors

	IC_{50} (μM)[a]				EC_{50} (μM)[a]
compd	[^{3}H]CPP	[^{3}H]AMPA	[^{3}H]MK-801	[^{3}H]kainic acid	cortical wedge
6[d]	4.5	>100	>100	>100	12[b]
(*S*)-**7**[e]	>100	0.02	>100	>100	3.5[c]
(*R*)-**7**[e]	>100	76	>100	>100	580[c]
8[f]	>100	>100	>100	>100	>1000
9[g]	>100	49	>100	>100	540[c]
10	>100	>100	>100	>100	>1000

[a] Mean of three to four experiments. [b] NMDA agonist. [c] AMPA agonist. [d] From ref 19. [e] From ref 23. [f] From ref 20. [g] From ref 21.

In Vitro Pharmacology

Activity at iGlu Receptors. In Table 1 the effects of the homologous series of 3-isoxazolol amino acids, AMAA (**6**)−**10**, at membrane-bound NMDA, AMPA, and kainic acid receptors are summarized. The earlier reported specific NMDA agonist effect of AMAA (**6**)[19] and the highly stereoselective AMPA agonist action of the *S*-form of AMPA (**7**)[23] are emphasized. With the exception of the very weak AMPA agonist effect of **9**,[21] the three higher homologues of AMPA tested did not show significant effect at iGlu receptors.

Activity at mGlu Receptors. The eight subtypes of mGlu receptors can be divided in three groups based on pharmacology, signal transduction pathway, and sequence homology: $mGlu_1$/$mGlu_5$, $mGlu_2$/$mGlu_3$, and $mGlu_4$/$mGlu_6$/$mGlu_7$/$mGlu_8$. In this study, we have chosen $mGlu_{1\alpha}$, $mGlu_2$, and $mGlu_{4a}$/$mGlu_6$ as representatives for these three groups. Initially, we tested the compounds depicted in Figure 1 as racemates or enantiomers, when available, at 1 mM concentration in the three cell lines. In $mGlu_{1\alpha}$-expressing cells, (*S*)-Glu increased the total level of inositol phosphates about 6-fold (Figure 2a) with an EC_{50} value of 19 ± 3 μM. However, none of the remaining compounds showed any significant agonist activity at $mGlu_{1\alpha}$. At the $mGlu_2$-expressing cell line, (*S*)-Glu inhibited the forskolin-stimulated cyclic AMP level to 10−20% of control levels (Figure 2b). Of all the tested compounds, only (*S*)-2-aminoadipic acid [(*S*)-**3**] displayed any significant agonist activity and was able to inhibit the forskolin-stimulated cyclic AMP level to the same extent as (*S*)-**2**, indicating full agonism (Figure 2b). When examined in greater detail (*S*)-**3** had an EC_{50} value of 35 ± 1 μM, some 3−4-fold higher than that of (*S*)-Glu (see Figure 3a and Table 2). This is in agreement with previously published results using $mGlu_2$-expressing baby hamster kidney cell. However, in that study, (*S*)-**3** was about 80 times weaker than (*S*)-Glu.[24] Finally, we tested the compounds at $mGlu_6$-expressing cells. (*S*)-Glu inhibited the forskolin-stimulated cyclic AMP level to 40−50% of control levels (Figure 2c). Of the tested compounds, (*S*)-**3**, (*S*)-**7**, and homo-AMPA (**8**), as well as compound **9**, were able to inhibit the forskolin-stimulated cyclic AMP formation to approximately the same extent as (*S*)-Glu (Figure 2c). Dose−response curves of these compounds revealed a rank order of potency of (*S*)-Glu > **8** ≥ (*S*)-**3** ≥ (*S*)-**7** ≥ **9** (Figure 3b and Table 2). The effect of (*S*)-**7** is in agreement with a previous report in which (*RS*)-**7** at 1 mM could inhibit 50% of the response.[9] On our cell line, (*S*)-**3** was some 7-fold weaker than (*S*)-Glu. (*S*)-**3** has previously been tested in $mGlu_4$-expressing BHK cells and found to be inactive even in 1 mM concentration.[24] Thus, there appears to be a disagreement between these two results, since both $mGlu_4$ and $mGlu_6$ belong to group III and previously have been shown to exhibit very similar pharmacology.

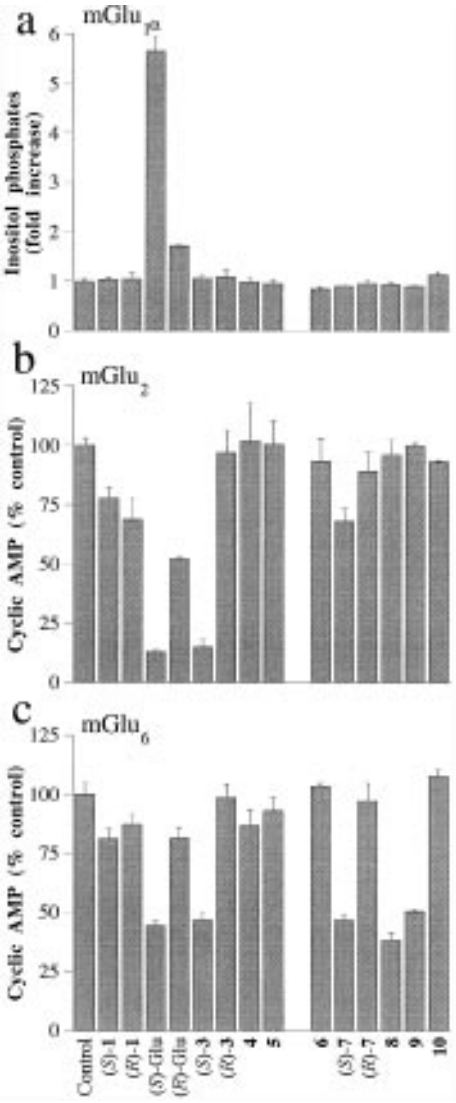


Figure 2. Agonist activities of the acidic amino acids and corresponding 3-isoxazolol bioisosteres at CHO cells expressing $mGlu_{1\alpha}$, $mGlu_2$, and $mGlu_6$. (a) $mGlu_{1\alpha}$-expressing cells were incubated with ligands at a concentration of 1 mM for 20 min. Total IP formation was determined by ion-exchange assay and the fold increase in IP level calculated compared to control cells (incubated in buffer only). (b and c) $mGlu_2$- and $mGlu_6$-expressing cells were incubated with ligands (1 mM) for 10 min in the presence of 10 μM forskolin, and cyclic AMP levels were measured by a RIA assay (Amersham). Cyclic AMP levels in control cells (incubated in buffer only) were set to 100%. Data are the means (±SD) of representative experiments performed in triplicate.

In order to investigate this apparent disagreement, we tested all compounds that had shown $mGlu_6$ activity in $mGlu_{4a}$-expressing cells. As seen in Figure 4, (*S*)-Glu inhibited the forskolin-stimulated cyclic AMP formation to 50−60% of control levels, whereas none of the other compounds tested, even at a concentration of 1 mM, had any measurable effect. Thus, the discrepancy appears to be the result of receptor subtype selectivity rather than a result of using different cell lines.

We also tested the compounds showing no significant agonist effect as antagonists at the mGlu receptors.

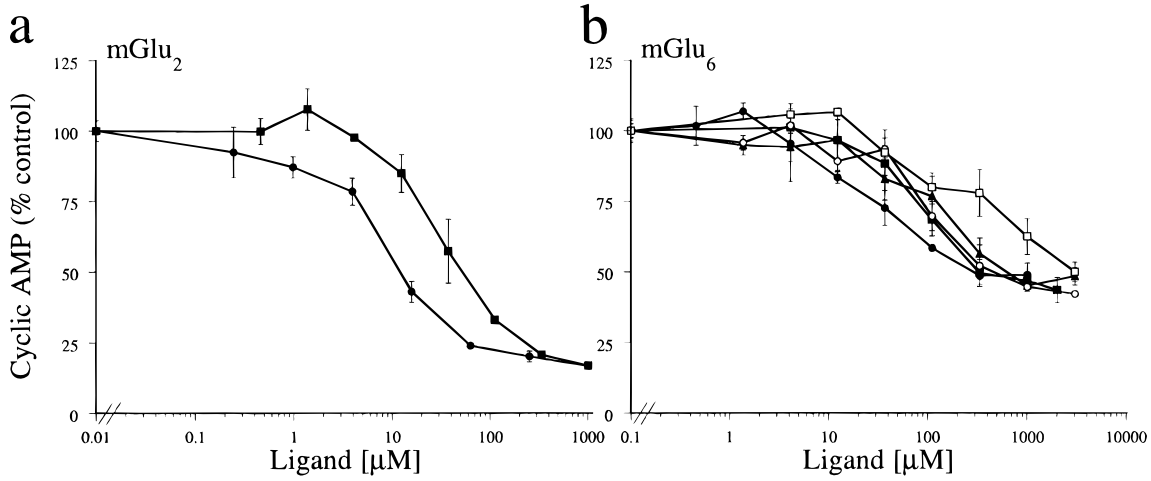


Figure 3. Dose−response curves for agonists at CHO cells expressing $mGlu_2$ (a) or $mGlu_6$ (b). Cells were incubated with glutamic acid (●), (*S*)-2-aminoadipic acid [(*S*)-**3**] (■), (*S*)-AMPA [(*S*)-**7**] (▲), homo-AMPA (**8**) (○), and compound **9** (□). For further details, see Figure 2.

Table 2. Agonist Activities of the Tested Ligands at Metabotropic Excitatory Amino Acid Receptors Expressed in CHO Cells

	EC_{50} (μM)[a]			
compd	$mGlu_{1\alpha}$	$mGlu_2$	$mGlu_{4a}$	$mGlu_6$
(*S*)-**1**	>1000	>1000	nd	>1000
(*R*)-**1**	>1000	>1000	nd	>1000
(*S*)-Glu	19 ± 3	8.5 ± 0.1	15 ± 6	20 ± 4
(*R*)-Glu	>1000	>1000	nd	>1000
(*S*)-**3**	>1000	35 ± 1	>3000	140 ± 35
(*R*)-**3**	>1000	>1000	nd	>1000
4	>1000	>1000	nd	>1000
5	>1000	>1000	nd	>1000
6	>1000	>1000	nd	>1000
(*S*)-**7**	>1000	>1000	>3000	220 ± 83
(*R*)-**7**	>1000	>1000	nd	>1000
8	>1000	>1000	>3000	82 ± 15
9	>1000	>1000	>3000	410 ± 110
10	>1000	>1000	nd	>1000

[a] Mean ± standard error of mean of at least two independent experiments.

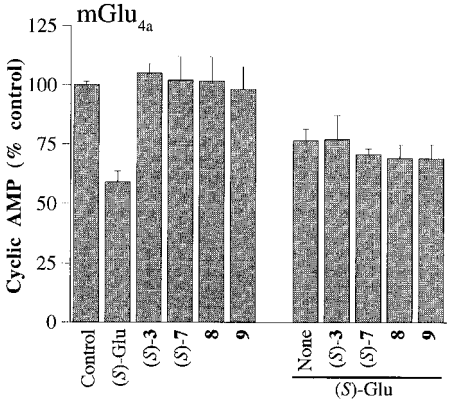


Figure 4. Agonist and antagonist activities at CHO cells expressing $mGlu_{4a}$ of ligands with significant agonist activities at $mGlu_6$-expressing cells. In agonist assays, cells were incubated with glutamic acid (1 mM) or test ligands (1 mM) for 10 min in the presence of 10 μM forskolin. In antagonist assays, cells were preincubated with ligand (1 mM) for 20 min and then incubated with ligand (1 mM) for 10 min in the presence of 50 μM (*S*)-Glu and 10 μM forskolin. For further details, see Figure 2.

However, as exemplified in Figure 4, none of these compounds had any significant antagonist effect.

Discussion

We have previously reported the design and pharmacology of specific agonists for iGlu receptors using the nonselective 3-isoxazolol amino acid ibotenic acid as a lead structure. Thus, the 3-isoxazolol bioisostere of aspartic acid (**1**), AMAA (**6**), is a specific NMDA agonist,[19] whereas the corresponding glutamic acid (**2**) analogue, AMPA (**7**), is a highly selective AMPA receptor agonist[16] (Figure 1). Within the framework of this EAA ligand design project, the higher homologue of AMPA (**7**), homo-AMPA (**8**), was shown to be completely inactive at iGlu receptors,[20] whereas the next homologue, compound **9**, is a very weak agonist at AMPA receptors[21] (Table 1).

In light of these observations, we decided to perform a comparative study of the homologous series of acidic amino acids ranging from aspartic acid (**1**) to 2-aminosuberic acid (**5**) and the respective 3-isoxazolol amino acids ranging from AMAA (**6**) to compound **10** (Figure 1). On all subtypes of mGlu receptors tested ($mGlu_{1\alpha}$, $mGlu_2$, $mGlu_{4a}$, and $mGlu_6$), (*S*)-Glu was the most potent agonist (Figures 2−4). In addition to (*S*)-Glu, the only member of this series of amino acids showing effects at mGlu receptors was (*S*)-2-aminoadipic acid [(*S*)-**3**], the higher homologue of (*S*)-Glu. (*S*)-**3** was shown to be an agonist at $mGlu_2$ and $mGlu_6$ but was inactive at $mGlu_{1\alpha}$ and $mGlu_{4a}$, indicating that this amino acid is capable of discriminating between the mGlu receptors of group III.

These observations represent a new facet of the complex pharmacology of **3**, the *R*-form of which shows NMDA antagonist effect,[2,25] whereas the *S*-form is an inhibitor of EAA uptake mechanism(s).[26,27] Furthermore, **3** has previously been shown to protect against kainic acid-induced neurotoxicity in the striatum[28] and the retina,[29] regions which express $mGlu_2$[30] and $mGlu_6$,[9] respectively. Finally, (*S*)-**3** has been shown[31] to antagonize the priming effect of quisqualic acid on depolarizations by (*R*)-Br-HIBO[32] or L-AP4.[33] The mechanism(s) underlying these neuroprotective and antipriming effects of **3** are not very well understood[29,31] and may involve activation of mGlu receptors. A prerequisite for testing this possible mechanism is the availability of selective agonists for the subtypes of mGlu receptors.

In this context, the testing of 3-isoxazolol amino acids on subtypes of mGlu receptors provided interesting

results. Thus, whereas (*S*)-AMPA [(*S*)-**7**] and homo-AMPA (**8**), as well as compound **9**, were inactive at $mGlu_{1\alpha}$, $mGlu_2$, and $mGlu_{4a}$, all of these 3-isoxazolol amino acids showed agonist effects at $mGlu_6$. Within this series of homologous amino acids, homo-AMPA (**8**) was the most potent compound, showing activity some 4 times weaker than (*S*)-Glu (Table 2). Since homo-AMPA (**8**) is the 3-isoxazolol analogue of **3** (Figure 1), this structure−activity relationship indicates that $mGlu_6$, in contrast to $mGlu_2$, is capable of accommodating the 3-isoxazolol unit as a bioisostere of the carboxyl group. It may be postulated that the slightly reduced conformational flexibility of homo-AMPA (**8**), as compared with **3**, can explain this selectivity.

In conclusion, homo-AMPA (**8**) has been shown to be a very selective and rather potent agonist at $mGlu_6$, being inactive at iGlu receptors and at $mGlu_{1\alpha}$, $mGlu_2$, and $mGlu_{4a}$. This observation is interesting in light of the pharmacology of structurally related 3-isoxazolol amino acids. Thus, the lower homologues AMAA (**6**) and AMPA (**7**) are highly selective agonists at NMDA and AMPA receptors, respectively, whereas the higher homologue **9** is a weak AMPA agonist, and the higher homologue **10** is inactive. Although homo-AMPA (**8**) is not as potent as L-AP4 ($EC_{50} = 0.9\ \mu M$ at $mGlu_6$),[9] we envisage that **8** will be a useful tool for studies of the pharmacology and physiology of $mGlu_6$ in the retina, since **8**, in contrast to L-AP4, shows selectivity within group III mGlu receptors.

Experimental Section

Chemistry. General Procedures. The structures of all new compounds were established by infrared (IR), ^1H and ^{13}C NMR spectroscopy, and elemental analyses. IR spectra were recorded on a Perkin-Elmer 781 grating infrared spectrophotometer. ^1H and ^{13}C NMR spectra were recorded at 200 and 50.32 MHz, respectively, on a Bruker AC-200 instrument, and chemical shift (δ) values are in parts per million with respect to an internal standard. Column chromatography (CC) was performed using silica gel 60, 0.063−0.200 mm (Merck). Analytical and preparative thin layer chromatography (TLC) was performed using silica gel 60 PF_{254}. Compounds containing the 3-isoxazolol unit were visualized on TLC plates using ultraviolet light and a $FeCl_3$ spraying reagent, and amino acids were visualized using a ninhydrin spraying reagent. Solvents were removed in vacuo by rotary evaporation at 15 mmHg. Melting points were determined in capillary tubes and are uncorrected. Elemental analyses were performed by Mr. G. Cornali, Microanalytical Laboratory, Leo Pharmaceutical Products, Denmark, and were within ±0.4% of the calculated values.

4-(2-Bromoethyl)-2-(methoxymethyl)-5-methylisoxazolin-3-one (12). A solution of compound **11**[22] (4.29 g, 30 mmol) in aqueous HBr (80 mL, 62%) was heated to 60 °C for 18 h in a sealed flask. The reaction mixture was cooled to 22 °C, and 1,3,5-trioxane (4.05 g, 45 mmol) was added. The flask was resealed and heated to 60 °C for 18 h. The reaction mixture was extracted with CH_2Cl_2 (4 × 100 mL), CH_3OH (200 mL) was added to the organic phase, and this reaction mixture was left at 22 °C for 30 min, after which CH_2Cl_2 (300 mL) and water (500 mL) were added. After extraction the organic phase was separated, and the aqueous phase was extracted with CH_2Cl_2 (100 mL). The combined CH_2Cl_2 phases were washed with water (2 × 250 mL), dried ($MgSO_4$), and evaporated to give crude **12** (8.4 g), which was subjected to CC [eluent: ethyl acetate−light petroleum (1:1)] to give **12** (6.8 g, 91%) as a colorless oil. ^1H NMR ($CDCl_3$, TMS): δ 2.29 (3H, s), 2.83 (2H, t, J = 6.5 Hz), 3.39 (3H, s), 3.61 (2H, t, J = 6.5 Hz), 5.14 (2H, s). ^{13}C NMR ($CDCl_3$): δ 11.6, 25.0, 30.9, 56.6, 74.7, 105.8, 166.9, 167.1. Anal. ($C_8H_{12}NO_3Br$) C, H, N, Br.

Methyl 2-Acetamido-2-(methoxycarbonyl)-4-[2-(methoxymethyl)-5-methyl-3-oxoisoxazolin-4-yl]butyrate (13) and 4-Ethenyl-2-(methoxymethyl)-5-methylisoxazolin-3-one (14). Dimethyl acetamidomalonate (5.20 g, 27.5 mmol) was dissolved in dry DMF (180 mL), and a suspension (60%) of sodium hydride (1.10 g, 27.5 mmol) in mineral oil was added. The reaction mixture was stirred for 1 h, and a solution of **12** (6.30 g, 25.2 mmol) in dry DMF (20 mL) was added. This reaction mixture was left at 22 °C for 14 days and then at 40 °C for 2 days. CH_3COOH (5 mL) was added, and the reaction mixture was evaporated. Water (200 mL) was added and the mixture extracted with CH_2Cl_2 (4 × 150 mL). The organic phase was dried ($MgSO_4$) and evaporated, and the product was subjected to CC [eluent: ethyl acetate−light petroleum (1:1)] to provide **13** as a crystalline product (5.55 g, 62%), mp 79−80 °C, and **14** as an oil (0.59 g, 14%). Data for **13**: ^1H NMR ($CDCl_3$, TMS): δ 2.09 (3H, s), 2.18 (2H, m), 2.21 (3H, s), 2.55 (2H, m), 3.40 (3H, s), 3.77 (6H, s), 5.11 (2H, s), 7.12 (1H, s). ^{13}C NMR ($CDCl_3$): δ 11.5, 15.4, 22.6, 30.9, 53.2, 56.8, 65.5, 75.0, 107.2, 166.0, 167.6, 168.0, 169.2. Anal. ($C_{15}H_{22}N_2O_8$) C, H, N. Data for **14**: ^1H NMR ($CDCl_3$, TMS): δ 2.31 (3H, s), 3.41 (3H, s), 5.15 (2H, s), 5.34 (1H, dd, J_{gem} = 3.1 Hz, J_{cis} = 10.3 Hz), 6.17 (1H, dd, J_{gem} = 3.1 Hz, J_{trans} = 17.6 Hz), 6.31 (1H, dd, J_{cis} = 10.3 Hz, J_{trans} = 17.6 Hz). ^{13}C NMR ($CDCl_3$): δ 11.7, 56.9, 74.9, 107.2, 116.7, 122.4, 165.7, 166.1. Anal. ($C_8H_{11}NO_3$) C, H, N.

2-Amino-4-(3-hydroxy-5-methylisoxazol-4-yl)butyric Acid (Homo-AMPA, 8). Compound **13** (1.85 g, 5.2 mmol) was refluxed in 1 M aqueous CF_3COOH (100 mL, 100 mmol) for 18 h. To the evaporated reaction product was added water (25 mL), and the mixture was evaporated. This procedure was repeated twice. A solution of the evaporated product in water (15 mL) was passed through a column containing Amberlite IRA-400 (OH^- form, 60 mL). The column was washed with water until the eluent was neutral. The product was eluted with 1 M aqueous CH_3COOH. The fractions containing product were collected and evaporated. The crude product was dissolved in water (50 mL) and the solution treated with activated carbon, filtered, and left at 5 °C for crystallization. The yield of homo-AMPA (**8**) was 451 mg (43%), mp 230 °C dec. ^1H NMR (D_2O, dioxane, 1 M NaOD): δ 1.68 (2H, m), 2.11 (3H, s), 2.17 (2H, t, J = 8.0 Hz), 3.22 (1H, t, J = 6.5 Hz). ^{13}C NMR (D_2O, dioxane, 1 M NaOD): δ 11.9, 18.2, 34.9, 56.5, 107.5, 166.6, 178.5, 183.1. Anal. ($C_8H_{12}N_2O_4 \cdot 0.25H_2O$) C, H, N.

Diethyl 1-Acetylpentane-1,5-dicarboxylate (16). Ethyl acetoacetate (5.0 g, 38.4 mmol) was added to a solution of sodium ethoxide prepared from sodium (883 mg, 38.4 mmol) and ethanol (100 mL), and the solution was heated to reflux. Ethyl 5-bromovalerate (8.82 g, 42.2 mmol) was slowly added together with sodium iodide (6.63 g, 42.2 mmol), and the mixture was refluxed for 18 h. The mixture was evaporated to dryness followed by distillation (0.1 mmHg, 200 °C) in a Kugelrohr apparatus to produce **16** (8.53 g, 86.0%). ^1H NMR ($CDCl_3$, TMS): δ 1.28 (6H, dt, J = 7.2, 1.8 Hz), 1.48−1.71 (4H, m), 1.86 (2H, quintet, J = 7.2 Hz), 2.23 (3H, s), 2.30 (2H, t, J = 7.2 Hz), 3.42 (1H, t, J = 7.2 Hz), 4.12 (4H, dq, J = 7.2, 1.8 Hz). ^{13}C NMR ($CDCl_3$): δ 13.9, 14.0, 24.4, 26.6, 27.5, 28.6, 33.7, 59.3, 60.0, 61.1, 169.5, 173.1, 203.1. Anal. ($C_{13}H_{22}O_5$) C, H.

Ethyl 5-(3-Hydroxy-5-methylisoxazol-4-yl)pentanoate (17). A solution of hydroxylammonium chloride (3.50 g, 50.3 mmol) in ethanol (20 mL) and water (1 mL) was added to a solution of sodium hydroxide (1.06 g, 26.4 mmol) in ethanol (20 mL) and water (1 mL) at 60 °C. This reaction mixture was filtered and, after cooling to −60 °C, added to a solution of **16** (6.50 g, 25.2 mmol) in ethanol (5 mL). Stirring was continued at −60 °C for 2 h. Acetone (1.85 mL, 25.2 mmol) was added to the reaction mixture followed by addition of a 80 °C solution of concentrated HCl (10 mL) and water (20 mL), additional stirring at 80 °C for 30 min, evaporation to dryness, extraction with CH_2Cl_2 (3 × 50 mL), addition of acetyl chloride (10 mL) in ethanol (90 mL), refluxing for 3 h, and evaporation to dryness followed by reevaporation three times with toluene. The residue was subjected to CC [eluent: toluene−ethyl acetate (1:1)] which produced **17** as a crystalline product (3.6

g, 63%), mp 57−58 °C. ^{1}H NMR ($CDCl_3$, TMS): δ 1.25 (3H, t, J = 6.8 Hz), 1.50−1.75 (4H, m), 2.25 (3H, s), 2.26−2.36 (4H, m), 4.12 (2H, q, J = 6.8 Hz), 11.71 (1H, s). ^{13}C NMR ($CDCl_3$): δ 11.2, 14.0, 20.4, 24.2, 28.0, 33.7, 60.0, 105.0, 165.3, 170.2, 173.3. Anal. ($C_{11}H_{17}NO_4$) H, N; C: calcd, 58.14; found, 58.89.

Ethyl 5-(3-Ethoxy-5-methylisoxazol-4-yl)pentanoate (18). Compound **17** (2.97 g, 13.9 mmol) was dissolved in a solution of sodium hydroxide (0.56 g, 13.9 mmol) in water (10 mL) followed by evaporation to dryness using an oil pump (0.05 mmHg). The residue was dissolved in dry DMF (10 mL) and cooled to −40 °C. Ethyl iodide (1.69 mL, 20.9 mmol) was added, and stirring was continued for 15 min at −40 °C. Further stirring at 22 °C for 30 min and evaporation to dryness followed by preparative TLC [eluent: toluene−ethyl acetate (1:1)] produced **18** (2.3 g, 65%) as an oil. ^{1}H NMR ($CDCl_3$, TMS): δ 1.25 (3H, t, J = 7.2 Hz), 1.40 (3H, t, J = 7.1 Hz), 1.45−1.72 (4H, m), 2.24 (3H, s), 2.25−2.36 (4H, m), 4.13 (2H, q, J = 7.1 Hz), 4.28 (2H, q, J = 7.2 Hz). ^{13}C NMR ($CDCl_3$): δ 11.3, 14.1, 14.5, 20.5, 24.2, 28.2, 33.8, 60.1, 65.1, 104.3, 165.2, 170.8, 173.4. Anal. ($C_{13}H_{21}NO_4$) C, H, N.

5-(3-Ethoxy-5-methylisoxazol-4-yl)pentanal (19). Under a nitrogen atmosphere, a solution of diisobutylaluminum hydride (1 M, 3.92 mL, 3.92 mmol) was added to a solution of **18** (400 mg, 1.56 mmol) in dry toluene (6.7 mL). Stirring was continued for 6 min, after which a saturated aqueous solution of sodium potassium tartrate (4.15 mL) was added to quench the reaction. Extraction with ether (4 × 25 mL) and evaporation to dryness. CC of the residue [eluent: toluene−ethyl acetate (3:1)] produced **19** (311 mg, 94%) as an oil. ^{1}H NMR ($CDCl_3$, TMS): δ 1.40 (3H, t, J = 7.1 Hz), 1.47−1.68 (4H, m), 2.24 (3H, s), 2.23−2.30 (2H, m), 2.47 (2H, dt, J = 1.6, 7.1 Hz), 4.28 (2H, q, J = 7.1 Hz), 8.76 (1H, t, J = 1.6 Hz). ^{13}C NMR ($CDCl_3$): δ 11.4, 14.6, 20.6, 21.3, 28.2, 43.4, 65.2, 104.2, 165.3, 170.8, 202.3. Anal. ($C_{11}H_{17}NO_3$) C, H, N.

5-[4-(3-Ethoxy-5-methylisoxazol-4-yl)butyl]hydantoin (20). Compound **19** (200 mg, 0.95 mmol), potassium cyanide (616 mg, 9.46 mmol), ammonium carbonate (473 mg, 4.73 mmol), and CH_3OH (50%, 3.5 mL) were refluxed for 16 h. Evaporation to dryness, extraction with ethyl acetate (3 × 10 mL), and reevaporation produced a crude product, which was purified by preparative TLC [eluent: toluene−ethyl acetate (1:4)] to give **20** (105 mg, 39%) as colorless crystals, mp 222.5−223.0 °C. ^{1}H NMR ($CDCl_3$, TMS): δ 1.39 (3H, t, J = 7.1 Hz), 1.43−1.98 (6H, m), 2.23 (3H, s), 2.27 (2H, t, J = 6.8 Hz), 4.09 (1H, dt, J = 2.9, 4.5 Hz), 4.29 (2H, q, J = 7.1 Hz), 6.38 (1H, s), 8.52 (1H, s). ^{13}C NMR ($CDCl_3$): δ 11.4, 14.6, 20.6, 24.2, 28.3, 31.3, 58.6, 65.3, 104.3, 157.3, 165.4, 171.0, 174.4. Anal. ($C_{13}H_{19}N_3O_4$) C, H, N.

2-Amino-6-(3-hydroxy-5-methylisoxazol-4-yl)hexanoic Acid (10). A solution of **20** (55 mg, 0.20 mmol) in HCl (6 M, 4 mL) was heated to 100 °C for 24 h in a sealed ampule. Evaporation of the solvent produced a crude product, which was subjected to preparative TLC [eluent: acetonitrile−acetic acid−water (8:1:1)] to give **10** (15 mg, 34%) after recrystallization from water, mp 198−199 °C dec. ^{1}H NMR (D_2O, dioxane, 1 M NaOD): δ 1.28−1.60 (4H, m), 1.82−1.99 (2H, m), 2.20 (3H, s), 2.23 (3H, s), 2.23 (2H, t, J = 7.0 Hz), 4.02 (1H, t, J = 6.3 Hz). ^{13}C NMR (D_2O, dioxane, 1 M NaOD): δ 11.7, 20.4, 24.3, 28.1, 30.3, 53.6, 67.4, 107.3, 169.4, 172.9. Anal. ($C_{10}H_{16}N_2O_4$) C, H, N.

Receptor Binding Assays. The membrane preparation used in the [^{3}H]-4-(3-phosphonoprop-1-yl)piperazine-2-carboxylic acid ([^{3}H]CPP) and [^{3}H]-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine ([^{3}H]MK-801) (two NMDA receptor antagonist radioligands), [^{3}H]AMPA, and [^{3}H]kainic acid binding assays was prepared according to the method of Ransom and Stec.[34] [^{3}H]CPP binding was studied following a published procedure,[35] where termination of the assays was accomplished using filtration through Whatman GF/B filters [presoaked in 0.1% poly(ethylenimine)] using a Brandell M-48R cell harvester rather than by centrifugation. [^{3}H]-AMPA binding was performed following a published procedure by Honoré and Nielsen.[36] [^{3}H]MK-801 binding to fully stimulated membranes was performed essentially as described earlier,[37] although the incubation time was increased from 1 to 4 h, and, furthermore, a concentration of radioactive ligand of 5 nM was used instead of 2 nM. [^{3}H]Kainic acid binding was performed as described by Braitman and Coyle[38] with the following modifications: The concentration of [^{3}H]kainic acid was 5 nM rather than 1 nM, and the reaction was terminated by filtration through Whatman GF/B filters followed by washing with ice cold 50 mM Tris-HCl buffer (2 × 5 mL, pH 7.1).

Electrophysiology in Vitro. A rat cortical slice preparation for testing EAAs described by Harrison and Simmonds[39] was used as modified by Wheatley.[40] Wedges (500 μm thick) of rat brain containing cerebral cortex and corpus callosum were placed with the corpus callosum on a wick of an Ag/AgCl electrode electrically insulated from the cortex part, which was placed between two layers of nappy liner and constantly perfused with a Mg^{2+}-free oxygenated Krebs solution (at room temperature). A reference electrode was placed in contact with the nappy liner, and the potential difference between the electrodes was recorded on an ABB SE120 chart recorder. Standard and test compounds were dissolved in the superfusion medium.

Cell Culture. The Chinese hamster ovary (CHO) cell lines expressing $mGlu_{1\alpha}$, $mGlu_2$, $mGlu_{4a}$, and $mGlu_6$ have been described previously.[8,9,13,41] They were maintained at 37 °C in a humidified 5% CO_2 incubator in DMEM which contained a reduced concentration of (*S*)-glutamine (2 mM) and was supplemented with 1% proline, penicillin (100 U/mL), streptomycin (100 mg/mL), and 10% dialyzed fetal calf serum (all GIBCO, Paisley, Scotland). Two days before assay 1.8×10^6 cells were divided into the wells of 12-well plates (PI assay) or 24-well plates (cyclic AMP assay).

Measurement of PI Hydrolysis and Cyclic AMP Formation. PI hydrolysis was measured as described previously.[42,43] Briefly, the cells were labeled with [^{3}H]inositol (1 μCi/mL) 24 h prior to the assay. For agonist assay, the cells were incubated with ligand dissolved in PBS−LiCl for 20 min, and agonist activity was determined by measurement of the level of ^{3}H-labeled mono-, bis-, and tris-inositol phosphates by ion-exchange chromatography. For antagonist assay, the cells were preincubated with the ligand dissolved in PBS−LiCl for 20 min prior to incubation with ligand and 10 μM (*S*)-Glu for 20 min. The antagonist activity was then determined as the inhibitory effect of the (*S*)-Glu-mediated response. The assay of cyclic AMP formation was performed as described previously.[42,43] Briefly, the cells were incubated for 10 min in PBS containing the ligand, 10 μM forskolin, and 1 mM 3-isobutyl-1-methylxanthine (IBMX) (both Sigma Chemicals, St. Louis, MO). The agonist activity was then determined as the inhibitory effect of the forskolin-induced cyclic AMP formation. For antagonist assay, the cells were preincubated with ligand dissolved in PBS containing 1 mM IBMX for 20 min prior to a 10 min incubation in PBS containing the ligand, (*S*)-Glu, 10 μM forskolin, and 1 mM IBMX. All experiments were performed at least twice in triplicate.

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References

(1) Choi, D. W.; Rothman, S. M. The role of glutamate neurotoxicity in hypoxic-ischemic neuronal death. *Annu. Rev. Neurosci.* **1990**, *13*, 171−182.
(2) Watkins, J. C.; Krogsgaard-Larsen, P.; Honoré, T. Structure-activity relationships in the development of excitatory amino acid receptor agonists and competitive antagonists. *Trends Pharmacol. Sci.* **1990**, *11*, 25−33.
(3) Bliss, T. V. P.; Collingridge, G. L. A synaptic model of memory: long-term potentiation in the hippocampus. *Nature* **1993**, *361*, 31−39.
(4) Nakanishi, S.; Masu, M. Molecular diversity and functions of glutamate receptors. *Annu. Rev. Biophys. Biomol. Struct.* **1994**, *23*, 319−348.
(5) Houamed, K. M.; Kuijper, J. L.; Gilbert, T. L.; Haldeman, B. A.; O'Hara, P. J.; Mulvihill, E. R.; Almers, W.; Hagen, F. S. Cloning, expression, and gene structure of a G protein-coupled glutamate receptor from rat brain. *Science* **1991**, *252*, 1318−1321.

(6) Masu, M.; Tanabe, Y.; Tsuchida, K.; Shigemoto, R.; Nakanishi, S. Sequence and expression of a metabotropic glutamate receptor. *Nature* **1991**, *349*, 760−765.
(7) Abe, T.; Sugihara, H.; Nawa, H.; Shigemoto, R.; Mizuno, N.; Nakanishi, S. Molecular characterization of a novel metabotropic glutamate receptor mGluR5 coupled to inositol phosphate/Ca2+ signal transduction. *J. Biol. Chem.* **1992**, *267*, 13361−13368.
(8) Tanabe, Y.; Masu, M.; Ishii, T.; Shigemoto, R.; Nakanishi, S. A family of metabotropic glutamate receptors. *Neuron* **1992**, *8*, 169−179.
(9) Nakajima, Y.; Iwakabe, H.; Akazawa, C.; Nawa, H.; Shigemoto, R.; Mizuno, N.; Nakanishi, S. Molecular characterization of a novel retinal metabotropic glutamate receptor mGluR6 with a high agonist selectivity for L-2-amino-4-phosphonobutyrate. *J. Biol. Chem.* **1993**, *268*, 11868−11873.
(10) Okamoto, N.; Hori, S.; Akazawa, C.; Hayashi, Y.; Shigemoto, R.; Mizuno, N.; Nakanishi, S. Molecular characterization of a new metabotropic glutamate receptor mGluR7 coupled to inhibitory cyclic AMP signal transduction. *J. Biol. Chem.* **1994**, *269*, 1231−1236.
(11) Duvoisin, R. M.; Zhang, C. X.; Ramonell, K. A novel metabotropic glutamate receptor expressed in the retina and olfactory bulb. *J. Neurosci.* **1995**, *15*, 3075−3083.
(12) Hayashi, Y.; Momiyama, A.; Takahashi, T.; Ohishi, H.; Ogawa-Meguro, R.; Shigemoto, R.; Mizuno, N.; Nakanishi, S. Role of a metabotropic glutamate receptor in synaptic modulation in the accessory olfactory bulb. *Nature* **1993**, *366*, 687−690.
(13) Tanabe, Y.; Nomura, A.; Masu, M.; Shigemoto, R.; Mizuno, N.; Nakanishi, S. Signal transduction, pharmacological properties, and expression patterns of two rat metabotropic glutamate receptors, mGluR3 and mGluR4. *J. Neurosci.* **1993**, *13*, 1372−1378.
(14) Nakanishi, S. Metabotropic glutamate receptors: Synaptic transmission, modulation, and plasticity. *Neuron* **1994**, *13*, 1031−1037.
(15) Knöpfel, T.; Kuhn, R.; Allgeier, H. Metabotropic glutamate receptors: Novel targets for drug development. *J. Med. Chem.* **1995**, *38*, 1417−1426.
(16) Krogsgaard-Larsen, P.; Honoré, T.; Hansen, J. J.; Curtis, D. R.; Lodge, D. New class of glutamate agonist structurally related to ibotenic acid. *Nature* **1980**, *284*, 64−66.
(17) Thomsen, C.; Bau, A.; Faarup, P.; Foged, C.; Kanstrup, A.; Suzdak, P. D. Effects of bromohomoibotenate on metabotropic glutamate receptors. *NeuroReport* **1994**, *5*, 2417−2420.
(18) Pellicciari, R.; Luneia, R.; Costantino, G.; Marinozzi, M.; Natalini, B.; Jakobsen, P.; Kanstrup, A.; Lombardi, G.; Moroni, F.; Thomsen, C. 1-Aminoindan-1,5-dicarboxylic acid: A novel antagonist at phospholipase C-linked metabotropic glutamate receptors. *J. Med. Chem.* **1995**, *38*, 3717−3719.
(19) Madsen, U.; Ferkany, J. W.; Jones, B. E.; Ebert, B.; Johansen, T. N.; Holm, T.; Krogsgaard-Larsen, P. NMDA receptor agonists derived from ibotenic acid. Preparation, neuroexcitation and neurotoxicity. *Eur. J. Pharmacol.* **1990**, *189*, 381−391.
(20) Madsen, U.; Frølund, B.; Lund, T. M.; Ebert, B.; Krogsgaard-Larsen, P. Design, synthesis and pharmacology of model compounds for indirect elucidation of the topography of AMPA receptor sites. *Eur. J. Med. Chem.* **1993**, *28*, 791−800.
(21) Christensen, I. T.; Reinhardt, A.; Nielsen, B.; Ebert, B.; Madsen, U.; Nielsen, E. Ø.; Brehm, L.; Krogsgaard-Larsen, P. Excitatory amino acid agonists and partial agonists. *Drug Des. Del.* **1989**, *5*, 57−71.
(22) Jacobsen, N.; Kolind-Andersen, H.; Christensen, J. Synthesis of 3-isoxazolols revisited. Diketene and β-ketoesters as starting materials. *Can. J. Chem.* **1984**, *62*, 1940−1944.
(23) Nielsen, B.; Fisker, H.; Ebert, B.; Madsen, U.; Curtis, D. R.; Krogsgaard-Larsen, P.; Hansen, J. J. Enzymic resolution of AMPA by use of α-chymotrypsin. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 107−114.
(24) Thomsen, C.; Hansen, L.; Suzdak, P. D. L-glutamate uptake inhibitors may stimulate phosphoinositide hydrolysis in baby hamster kidney cells expressing mGluR1a via heteroexchange with L-glutamate without direct activation of mGluR1a. *J. Neurochem.* **1994**, *63*, 2038−2047.
(25) Evans, R. H.; Francis, A. A.; Hunt, K.; Oakes, D. J.; Watkins, J. C. Antagonism of excitatory amino acid-induced responses and of synaptic excitation in the isolated spinal cord of the frog. *Br. J. Pharmacol.* **1979**, *67*, 591−603.
(26) Ferkany, J.; Coyle, J. T. Heterogeneity of sodium-dependent excitatory amino acid uptake mechanisms in rat brain. *J. Neurosci. Res.* **1986**, *16*, 491−503.
(27) Anderson, K. J.; Monaghan, D. T.; Bridges, R. J.; Tavoularis, A. L.; Cotman, C. W. Autoradiographic characterization of putative excitatory amino acid transport sites. *Neuroscience* **1990**, *38*. 311−322.
(28) McBean, G. J. Intrastriatal injection of DL-α-aminoadipate reduces kainate toxicity in vitro. *Neuroscience* **1990**, *34*, 225−234.
(29) Kleinschmidt, J.; Zucker, C. L.; Yazulla, S. Neurotoxic action of kainic acid in the isolated toad and goldfish retina: II. Mechanism of action. *J. Comp. Neurol.* **1986**, *254*, 196−208.
(30) Testa, C. M.; Standaert, D. G.; Young, A. B.; Penny, J. B. Metabotropic glutamate receptor mRNA expression in the basal ganglia of the rat. *J. Neurosci.* **1994**, *14*, 3005−3018.
(31) Harris, E. W. L-α-aminodipate antagonizes the priming effect of quisqualate in hippocampal slices. *Eur. J. Pharmacol.* **1989**, *161*, 107−109.
(32) Madsen, U.; Ebert, B.; Hansen, J. J.; Krogsgaard-Larsen, P. The non-depolarizing D-form of bromohomoibotenic acid (Br-HIBO) enhances depolarizations by L-Br-HIBO or quisqualate. *Eur. J. Pharmacol.* **1993**, *230*, 383−386.
(33) Whittemore, E. R.; Koerner, J. F. Novel recognition site for L-quisqualate sensitizes neurons to depolarization by L-2-amino-4-phosphonobutanote (L-AP4). *Brain Res.* **1989**, *489*, 146−156.
(34) Ransom, R. W.; Stec, N. L. Cooperative modulation of [^{3}H]MK-801 binding to the *N*-methyl-D-aspartate receptor ion channel complex by L-glutamate, glycine and polyamines. *J. Neurochem.* **1988**, *51*, 830−836.
(35) Murphy, D. E.; Schneider, J.; Boehm, C.; Lehmann, J.; Williams, K. Binding of [^{3}H]3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid to rat membranes. A selective high affinity ligand for N-methyl-D-aspartate receptors. *J. Pharmacol. Exp. Ther.* **1987**, *240*, 778−783.
(36) Honoré, T.; Nielsen, M. Complex structure of quisqualate-sensitive glutamate receptors in rat cortex. *Neurosci. Lett.* **1985**, *54*, 27−32.
(37) Foster, A. C.; Wong, E. H. F. The novel anticonvulsant MK-801 binds to the activated state of the N-methyl-D-aspartate receptor in rat brain. *Br. J. Pharmacol.* **1987**, *91*, 403−409.
(38) Braitman, D. J.; Coyle, J. T. Inhibition of [^{3}H]Kainic acid receptor binding by divalent cations correlates with ion affinity for the calcium channel. *Neuropharmacology* **1987**, *26*, 1247−1251.
(39) Harrison, N. L.; Simmonds, M. A. Quantitative studies on some antagonists of N-methyl-D-aspartate in slices of rat cerebral cortex. *Br. J. Pharmacol.* **1985**, *84*, 381−391.
(40) Wheatley, P. L. A simple method for recording excitatory amino acid-evoked depolarizations of rat cortex in vitro. *Br. J. Pharmacol.* **1986**, *87*, 159P.
(41) Aramori, I.; Nakanishi, S. Signal transduction and pharmacological characteristics of a metabotropic glutamate receptor, mGluR1, in transfected CHO cells. *Neuron* **1992**, *8*, 757−765.
(42) Hayashi, Y.; Tanabe, Y.; Aramori, I.; Masu, M.; Shigemoto, K.; Ohfune, Y.; Nakanishi, S. Agonist analysis of 2-(carboxycyclopropyl)glycine isomers for cloned metabotropic glutamate receptor subtypes expressed in Chinese hamster ovary cells. *Br. J. Pharmacol.* **1992**, *107*, 539−543.
(43) Hayashi, Y.; Sekiyama, N.; Nakanishi, S.; Jane, D. E.; Sunter, D. C.; Birse, E. F.; Udvarhelyi, P. M.; Watkins, J. C. Analysis of agonist and antagonist activities of phenylglycine derivatives of different cloned metabotropic glutamate receptor subtypes. *J. Neurosci.* **1994**, *14*, 3370−3377.

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