Novel Class of Amino Acid Antagonists at Non-*N*-methyl-D-aspartic Acid Excitatory Amino Acid Receptors. Synthesis, in Vitro and in Vivo Pharmacology, and Neuroprotection

Povl Krogsgaard-Larsen,*,† John W. Ferkany,‡ Elsebet Ø. Nielsen,† Ulf Madsen,† Bjarke Ebert,† Jørgen S. Johansen,† Nils H. Diemer,§ Torben Bruhn,§ David T. Beattie,⊥ and David R. Curtis⊥

PharmaBiotec Research Center, Department of Organic Chemistry, The Royal Danish School of Pharmacy, DK-2100 Copenhagen, Denmark, NOVA Pharmaceutical Corporation, CNS Research, Baltimore, Maryland 21224, Institute of Neuropathology, University of Copenhagen, DK-2100 Copenhagen, Denmark, and Neuropharmacology Research Group, Division of Neuroscience, The Australian National University, Canberra, ACT 2601, Australia. *Received April 2, 1990*

The isoxazole amino acid 2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid (AMPA) (1), which is a highly selective agonist at the AMPA subtype of excitatory amino acid (EAA) receptors, has been used as a lead for the development of two novel EAA receptor antagonists. One of the compounds, 2-amino-3-[3-(carboxymethoxy)-5-methylisoxazol-4-yl]propionic acid (AMOA, 7), was synthesized via O-alkylation by ethyl chloroacetate of the amino acid protected AMPA derivative 4. The other compound, 2-amino-3-[2-(3-hydroxy-5-methylisoxazol-4-yl)-methyl-5-methyl-3-oxoisoxazolin-4-yl]propionic acid (AMNH, 14) was synthesized with use of 4-(chloromethyl)-3-methoxy-5-methylisoxazole (8) as the starting material. The intermediate 4-(chloromethyl)-2-(3-methoxy-5-methylisoxazol-4-yl)methyl-5-methylisoxazolin-3-one (11) was converted into the acetamidomalonate (12), which was stepwise deprotected to give 14. Compounds 7 and 14 were stable in aqueous solution at pH values close to physiological pH. Neither 7 nor 14 showed detectable affinities for the receptor, ion channel, or modulatory sites of the *N*-methyl-D-aspartic acid (NMDA) receptor complex. Quantitative receptor autoradiographic and conventional binding techniques were used to study the affinities of 7 and 14 for non-NMDA receptor sites. Both compounds were inhibitors of the binding of [^{3}H]AMPA (IC_{50} = 90 and 29 μM, respectively). Compounds 14 and 7 were both very weak inhibitors of the high-affinity binding of radioactive kainic acid ([^{3}H]KAIN). Compound 14, but not 7, was, however, shown to be an inhibitor of low-affinity [^{3}H]KAIN binding (IC_{50} = 40 μM) as determined in the presence of 100 mM calcium chloride. In the rat cortical slice preparation, 7 was shown to antagonize excitation induced by 1 with some selectivity, whereas 14 proved to be a rather selective antagonist of KAIN-induced excitation. Both antagonists showed very weak effects on the excitatory effects of NMDA. Compound 7 was a poor antagonist of excitation by quisqualic acid (2), whereas 14 did not affect excitation by this nonselective AMPA receptor agonist. On cat spinal neurones, both 7 and 14 reduced excitations by 1 and KAIN, but, again, the excitatory effects of 2 were much less sensitive. Compound 14 and, in particular, 7 effectively protected rat striatal neurones against the neurotoxic effects of KAIN, whereas the toxic effects of 1 were reduced only by 7. Neither antagonist showed protection against the cell damage caused by intrastriatal injection of the NMDA agonist quinolinic acid. Taken together, these results are consistent with 7 and 14 being fairly selective competitive antagonists for AMPA and KAIN receptors, respectively, in rat cortical tissues.

The acidic amino acids (*S*)-aspartic acid (Asp) and, in particular, (*S*)-glutamic acid (Glu) are generally accepted to be the major excitatory amino acid (EAA) neurotransmitters in the mammalian central nervous system (CNS).[1-7] Hyperactivity of central EAA neuronal pathways has been associated with the etiology of certain neurodegenerative diseases such as status epilepticus, Huntington's chorea, and dementia of the Alzheimer type.[8-12] Furthermore, the neuronal degeneration observed after ischemia following stroke, hypoxia, or hypoglycemia may also be due to prolonged and excessive stimulation of EAA receptors.[8,12] In light of these observations there are pharmacological and therapeutic interests in compounds capable of blocking subtypes of central EAA receptors.

Central EAA receptors are subdivided into three main classes:[1-7] (1) *N*-methyl-D-aspartic acid (NMDA) receptors at which NMDA and quinolinic acid are agonists and 2-amino-5-phosphonopentanoic acid and 3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid (CPP) selective competitive antagonists, whereas 10,11-dihydro-5-methyl-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine (MK-801)[13] is a noncompetitive NMDA antagonist acting at a site near or in the ion channel operated by the NMDA receptor.[14] The NMDA receptor complex comprises a strychnine-insensitive binding site for glycine assumed to exert a modulatory function at this receptor subtype;[14] (2) 2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid (AMPA) receptors, at which AMPA (1)[15] is a highly se-

* Address correspondence to: Professor Povl Krogsgaard-Larsen, Department of Organic Chemistry, The Royal Danish School of Pharmacy, 2 Universitetsparken, DK-2100 Copenhagen Ø, Denmark.

† The Royal Danish School of Pharmacy.

‡ NOVA Pharmaceutical Corporation.

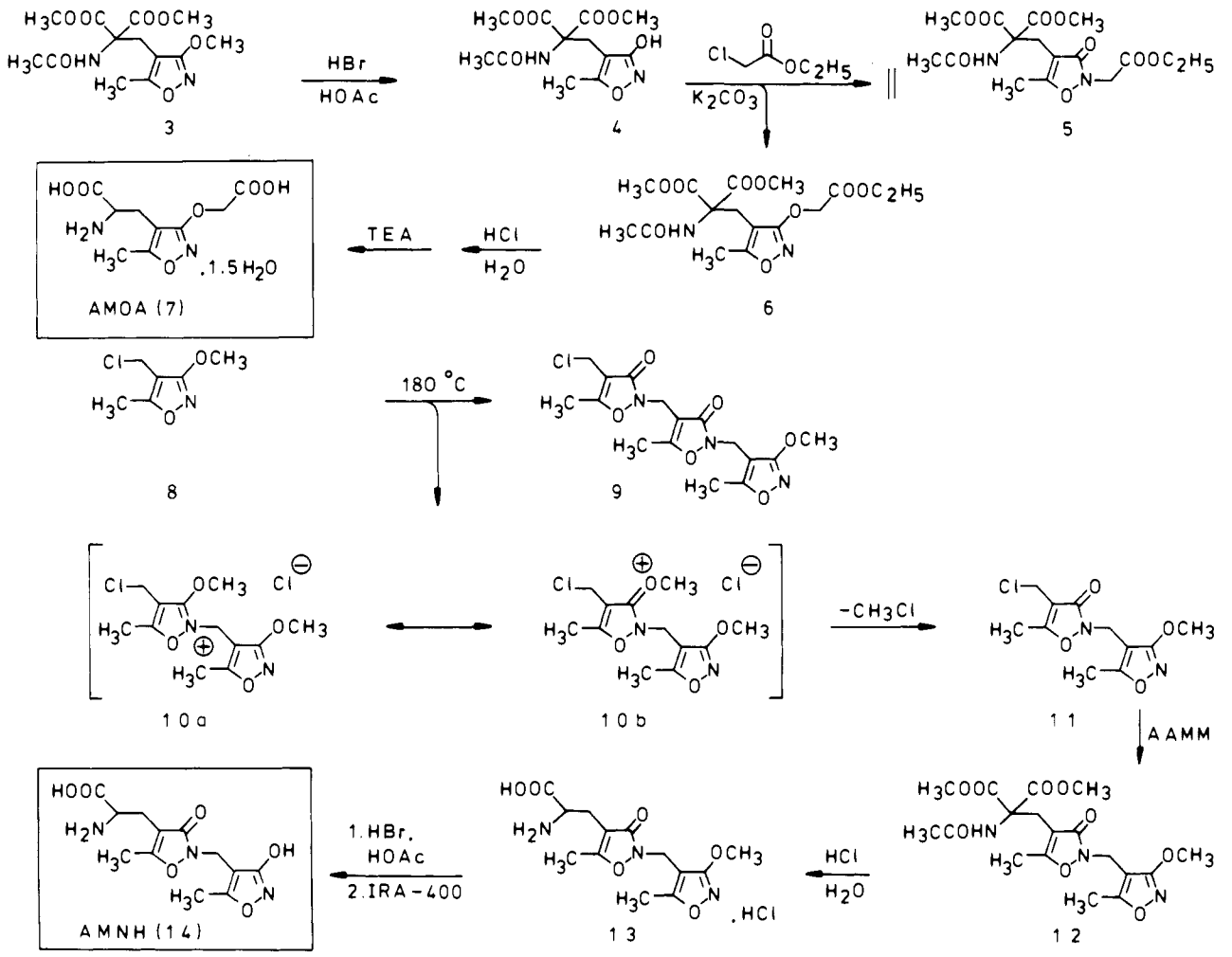
§ University of Copenhagen.

⊥ The Australian National University.

(1) Watkins, J. C.; Evans, R. H. *Annu. Rev. Pharmacol. Toxicol.* **1981**, *21*, 165.
(2) Foster, A. C.; Fagg, G. E. *Brain Res. Rev.* **1984**, *7*, 103.
(3) Johnson, R. L.; Koerner, J. F. *J. Med. Chem.* **1988**, *31*, 2057.
(4) Shinozaki, H. *Prog. Neurobiol.* **1988**, *30*, 399.
(5) Honorē, T. *Med. Res. Rev.* **1989**, *9*, 1.
(6) Hansen, J. J.; Krogsgaard-Larsen, P. *Med. Res. Rev.* **1990**, *10*, 55.
(7) Watkins, J. C.; Krogsgaard-Larsen, P.; Honorē, T. *Trends Pharmacol. Sci.* **1990**, *11*, 25.
(8) Rothman, S. M.; Olney, J. W. *Ann. Neurol.* **1986**, *19*, 105.
(9) Greenamyre, J. T. *Arch. Neurol.* **1986**, *43*, 1058.
(10) Greenamyre, J. T.; Maragos, W. F.; Albin, R. L.; Penney, J. B.; Young, A. B. *Prog. Neuro-Psychopharmacol. Biol. Psychiat.* **1988**, *12*, 421.
(11) Bridges, R. J.; Geddes, J. W.; Monaghan, D. T.; Cotman, C. W. In *Excitatory Amino Acids in Health and Disease*; Lodge, D., Ed.; J. Wiley and Sons: Chichester, England, 1988; p 321.
(12) Lodge, D., Ed. *Excitatory Amino Acids in Health and Disease*; J. Wiley and Sons: Chichester, England, 1988.
(13) Thompson, W. J.; Anderson, P. S.; Britcher, S. F.; Lyle, T. A.; Thies, J. E.; Magill, C. A.; Varga, S. L.; Schwering, J. E.; Lyle, P. A.; Christy, M. E.; Evans, B. E.; Colton, C. D.; Holloway, M. K.; Springer, J. P.; Hirshfield, J. M.; Ball, R. G.; Amato, J. S.; Larsen, R. D.; Wong, E. H. F.; Kemp, J. A.; Tricklebank, M. D.; Singh, L.; Oles, R.; Priestly, T.; Marshall, G. R.; Knight, A. R.; Middlemiss, D. N.; Woodruff, G. N.; Iversen, L. L. *J. Med. Chem.* **1990**, *33*, 789.
(14) Monaghan, D. T.; Bridges, R. J.; Cotman, C. W. *Ann. Rev. Pharmacol. Toxicol.* **1989**, *29*, 365.
(15) Hansen, J. J.; Krogsgaard-Larsen, P. *J. Chem. Soc., Perkin Trans. 1* **1980**, 1826.

0022-2623/91/1834-0123$02.50/0

Scheme I



lective agonist[16] and the naturally occurring amino acid quisqualic acid (**2**) a nonselective agonist.[1,7] AMPA receptors are effectively but nonselectively blocked by a number of quinoxaline derivatives, including 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX)[17] and 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo(*f*)quinoxaline (NBQX), some of which, notably NBQX, show selective affinity for AMPA receptor sites in binding studies;[5,7,18] (3) kainic acid (KAIN) receptors, which are selectively activated by KAIN[19] and effectively blocked by CNQX[17] and NBQX.[18]

The physiological relevance of high-affinity binding sites for [^{3}H]AMPA and [^{3}H]KAIN, which show different inhibitor selectivities,[20,21] regional distributions in the CNS,[22] and molecular target sizes,[23] is unclear.[14] Accumulating evidence supports the contention that low-affinity states of AMPA and KAIN receptors are functionally important,[5,14,24] and it has been proposed that AMPA receptors mediate, at least some of, the effects of KAIN.[1,18,24,25] There is a need for selective antagonists at AMPA and KAIN receptors, and compounds capable of blocking selectively low-affinity states of these receptors would be of major pharmacological interest.

We envisage that the selectivity of AMPA (**1**) as an AMPA receptor agonist is associated with the rigid and planar structure of the anionic 3-isoxazolyl unit as established by X-ray crystallography[26,27] (Figure 1). This heterocyclic nucleus is a bioisostere of the ω-carboxyl group of Glu.[6,16] These structural characteristics of **1** are only to a limited extent shared by the structurally related amino acid **2**.[28] The conformational mobility and lack of coplanarity of the heterocyclic ring of **2** may be of some importance for the ability of this amino acid to interact not only with AMPA, but also with NMDA and KAIN receptor sites[14,20,29] and with an EAA-associated enzyme[30] and transport site.[31]

(16) Krogsgaard-Larsen, P.; Honoré, T.; Hansen, J. J.; Curtis, D. R.; Lodge, D. *Nature (London)* **1980**, *284*, 64.
(17) Honoré, T.; Davies, S. N.; Drejer, J.; Fletcher, E. J.; Jacobsen, P.; Lodge, D.; Nielsen, F. E. *Science* **1988**, *241*, 701.
(18) Sheardown, M. J.; Nielsen, E. Ø.; Hansen, A. J.; Jacobsen, P.; Honoré, T. *Science* **1990**, *247*, 571.
(19) Coyle, J. T. *J. Neurochem.* **1983**, *41*, 1.
(20) Krogsgaard-Larsen, P.; Honoré, T. *Trends Pharmacol. Sci.* **1983**, *4*, 31.
(21) Ross, S. M.; Roy, D. N.; Spencer, P. S. *J. Neurochem.* **1989**, *53*, 710.
(22) Monaghan, D. T.; Holets, V. R.; Toy, D. W.; Cotman, C. W. *Nature (London)* **1983**, *306*, 176.
(23) Honoré, T.; Nielsen, M. *Neurosci. Lett.* **1985**, *54*, 27.
(24) Braitman, D. J.; Coyle, J. T. *Neuropharmacology* **1987**, *26*, 1247.
(25) Pin, J.-P.; Van Cliet, B. J.; Bockaert, J. *Eur. J. Pharmacol.-Mol. Pharmacol. Sect.* **1989**, *172*, 81.
(26) Honoré, T.; Lauridsen, J. *Acta Chem. Scand.* **1980**, *B34*, 235.
(27) Krogsgaard-Larsen, P.; Brehm, L.; Johansen, J. S.; Vinzents, P.; Lauridsen, J.; Curtis, D. R. *J. Med. Chem.* **1985**, *28*, 673.
(28) Jackson, D. E.; Bycroft, B. W.; King, T. J. *J. Comput.-Aided Mol. Design* **1988**, *2*, 321.
(29) Nielsen, E. Ø.; Madsen, U.; Schaumburg, K.; Brehm, L.; Krogsgaard-Larsen, P. *Eur. J. Med. Chem.* **1986**, *21*, 433.
(30) Robinson, M. B.; Blakely, R. D.; Coutou, R.; Coyle, J. T. *J. Biol. Chem.* **1987**, *262*, 14498.

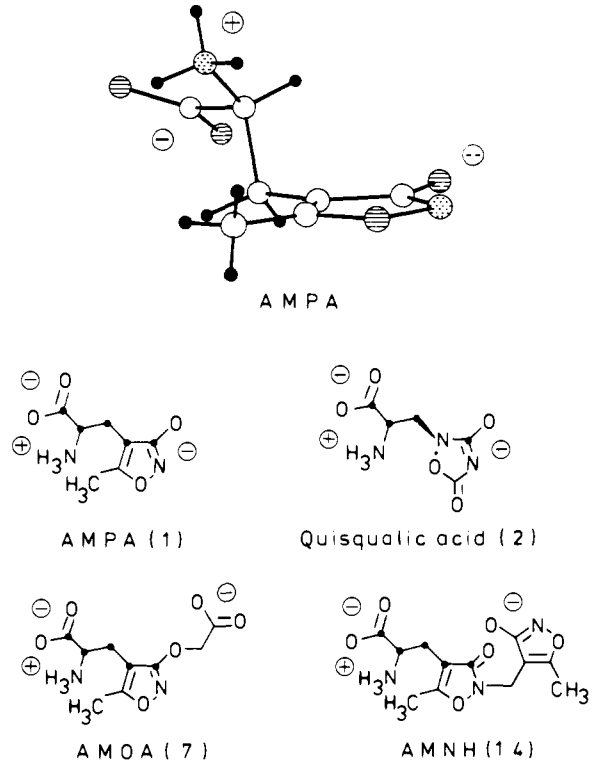


Figure 1. Illustration of the structure of AMPA (1) as determined by using X-ray crystallographic techniques[26,27] and a comparison of the molecules of 1, quisqualic acid (QUIS, 2), AMOA (7), and AMNH (14).

Using 1 as a lead structure in attempts to design antagonists selective for non-NMDA receptors we have now synthesized and tested the two compounds 2-amino-3-[3-(carboxymethoxy)-5-methylisoxazol-4-yl]propionic acid (AMOA, 7) and 2-amino-3-[2-(3-hydroxy-5-methylisoxazol-4-yl)-methyl-5-methyl-3-oxoisoxazolin-4-yl)]propionic acid (AMNH, 14) as potential EAA antagonists.

Results

Synthesis. Compound 3, an intermediate for the synthesis of 1,[15] was selectively deprotected to give the 3-isoxazolol 4 (Scheme I). Reactions of 3-isoxazolol derivatives with alkyl halides under basic conditions normally give mixtures of the isomeric 3-alkoxyisoxazoles and 2-alkylisoxazoline-3-ones.[32,33] However, only the O-alkylated compound 6 could be isolated in a pure state from the reaction mixture obtained by treatment of 4 with ethyl chloroacetate. Attempts to detect compound 5 in or to isolate it from this relatively complex reaction mixture by using HPLC techniques were unsuccessful. Deprotection of 6 under acidic conditions gave AMOA (7), which was isolated in the zwitterionic form.

The key step in the preparation of AMNH (14) was the conversion of 4-(chloromethyl)-3-methoxy-5-methylisoxazole (8) into 11. Heating of 8 at 180 °C for 3 h gave 11 in low yield (12%) accompanied by the formation of very small amounts (3%) of the corresponding "trimer" 9 (Scheme I). Several attempts to increase the yield of 11 by alteration of the reaction conditions were unsuccessful. Thus, heating of 8 at higher temperatures or for longer periods of time decreased and increased, respectively, the yields of 11 and 9. Similarly, heating of 8 in the presence of small amounts of thionyl chloride or reflux of solutions of 8 in DMF in the presence of lithium chloride gave 11 in very low yields. The formation of 11 (and also 9) from 8 is assumed to proceed via the N-alkylated intermediate **10a**, isomerization of **10a** to **10b**, and cleavage of the methyloxonium group of **10b** by nucleophilic attack of a chloride ion. Conversion of 11 into the acetamidomalonate 12 and stepwise deprotection of this intermediate through the 3-methoxyisoxazole amino acid 13 gave 14, which, like 7, was most conveniently isolated in the zwitterionic form.

The structures of all new compounds were established on the basis of spectroscopic methods and supported by elemental analyses.

In order to test the chemical stability of 7 and 14, solutions of these compounds in a 50 mM phosphate buffer (pH 7.4) adjusted to an ionic strength of 0.5 by addition of potassium chloride were left at room temperature for 2 weeks. On the basis of TLC analyses of these solutions during this period, no degradation products sensitive to ninhydrin or visible in UV light were formed in detectable amounts.

Receptor Binding. AMOA (7) and AMNH (14) were tested in a number of receptor binding assays. Neither compound showed detectable effects on the binding of [^{3}H]CPP, [^{3}H]MK-801, or [^{3}H]glycine indicating lack of affinity of 7 or 14 for these particular receptor, channel, or modulatory sites of the NMDA receptor complex. Furthermore, 7 and 14 did not significantly affect the binding of [^{3}H]GABA to $GABA_A$ or $GABA_B$ receptor sites.

In the [^{3}H]AMPA binding assay containing the chaotropic isothiocyanate ion (100 mM) both high- and low-affinity stages of AMPA receptor sites can be detected.[5,23,34] Under these conditions, AMOA (7) (IC_{50} = 90 μM) and AMNH (14) (IC_{50} = 29 μM) were inhibitors of [^{3}H]AMPA binding.

In routine [^{3}H]KAIN binding assays (no calcium chloride added) only high-affinity KAIN receptor sites are detected.[14,24,35] Both 7 and 14, tested at 100 μM concentrations, were very weak inhibitors of high-affinity [^{3}H]-KAIN receptor binding showing 36% and 24% inhibition, respectively. In the presence of high concentrations of calcium chloride only low-affinity [^{3}H]KAIN can be detected in rat brain membranes.[5] Under these assay conditions, the affinity of 14 for KAIN receptor sites was substantially increased (IC_{50} = 40 μM), whereas addition of 100 mM calcium chloride did not significantly change the affinity of 7 in the [^{3}H]KAIN binding assay.

The interactions of 7 and 14 with high- and low-affinity [^{3}H]AMPA binding sites (in the presence of 100 mM potassium isothiocyanate) and with low-affinity [^{3}H]KAIN binding sites (in the presence of 100 mM calcium chloride) were studied in further detail. The effects of 7 and 14 on the binding of [^{3}H]AMPA are illustrated in Figure 2. The shifts to the right of these binding curves suggest that 7 and 14 inhibit the binding of [^{3}H]AMPA in a competitive fashion. The significance of the slightly different slopes of the binding curves in the presence of 7 or 14 (Figure 2) is at present unclear.

The binding data summarized in Figure 2 were subjected to Scatchard analyses. In agreement with earlier findings,[5,23,34] [^{3}H]AMPA binding gave a curvilinear Scatchard

(31) Pin, J.-P.; Bockaert, J.; Rēcasens, M. *FEBS Lett.* **1984**, *175*, 31.
(32) Sauerberg, P.; Larsen, J.-J.; Falch, E.; Krogsgaard-Larsen, P. *J. Med. Chem.* **1986**, *29*, 1004.
(33) Sauerberg, P.; Falch, E.; Meier, E.; Lembøl, H. L.; Krogsgaard-Larsen, P. *J. Med. Chem.* **1988**, *31*, 1312.
(34) Nielsen, E. Ø.; Drejer, J.; Cha, J.-H. J.; Young, A. B.; Honorē, T. *J. Neurochem.* **1990**, *54*, 686.
(35) Honorē, T.; Drejer, J.; Nielsen, M. *Neurosci. Lett.* **1986**, *65*, 47.
(36) Honorē, T.; Lauridsen, J.; Krogsgaard-Larsen, P. *J. Neurochem.* **1982**, *38*, 173.
(37) Falch, E.; Krogsgaard-Larsen, P. *J. Neurochem.* **1982**, *38*, 1123.

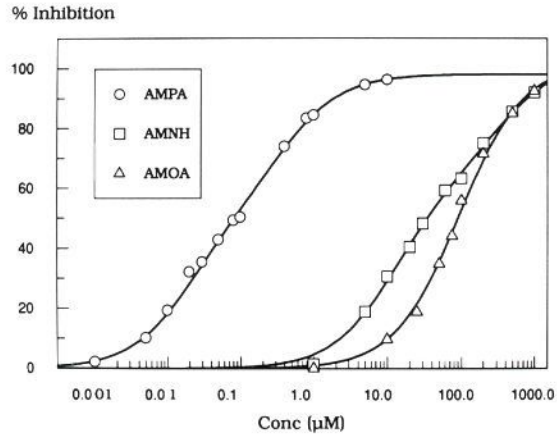


Figure 2. Binding of [^{3}H]AMPA in the presence of various concentrations of AMPA (**1**), AMOA (**7**), or AMNH (**14**). Each point is the result of at least three independent experiments. The binding assays were performed as described in the Experimental Section. The data illustrated in this figure were used for Scatchard analyses, from which the data summarized in Table I were derived.

Table I. Affinities of AMPA (**1**), AMOA (**7**), and AMNH (**14**) for AMPA Receptor Sites

	[^{3}H]AMPA receptor binding[a]			
	high-affinity		low-affinity	
compd	IC_{50}, μM	percentage of total binding	IC_{50}, μM	percentage of total binding
AMPA (**1**)	0.019	51	0.364	46
AMOA (**7**)	88	55	98	46
AMNH (**14**)	12	62	335	40

[a] The Scatchard analyses were performed by following a published procedure[36] on the basis of the displacement data summarized in Figure 2. IC_{50} values were calculated as described earlier.[37] As shown in Figure 2, the compounds were tested in the following concentration ranges: **1** (0.001–10 μM), **7** (1–1000 μM), and **14** (1–1000 μM).

plot, which could be resolved into two binding affinities (Table I). In displacement studies **7** was shown to interact with high- and low-affinity [^{3}H]AMPA binding sites with approximately equal affinities, whereas **14** was shown to bind much more tightly to high-affinity than to low-affinity AMPA receptor sites.

Dose–response curves for the inhibitory effects of **14** and **7** on low-affinity [^{3}H]KAIN binding are depicted in Figure 3. As mentioned earlier, **14** was shown to be a moderately potent inhibitor of this component of [^{3}H]KAIN receptor binding, whereas **7** was much weaker. Due to this very low affinity of **7**, a dose–response curve could not be produced for this compound (Figure 3). Addition of increasing concentrations of **14** to the test system shifted the [^{3}H]-KAIN binding curve to the right. The displacement curves using KAIN and **14** are, however, not parallel, suggesting that [^{3}H]KAIN and AMNH do not compete for binding to the low-affinity site in a strictly competitive manner. These aspects are at present under further investigation.

Receptor Autoradiography. In vitro radioligand autoradiography has emerged as a powerful technique,[38] which has been extensively used for studies of EAA receptors in the CNS.[39,40] It is generally believed that the

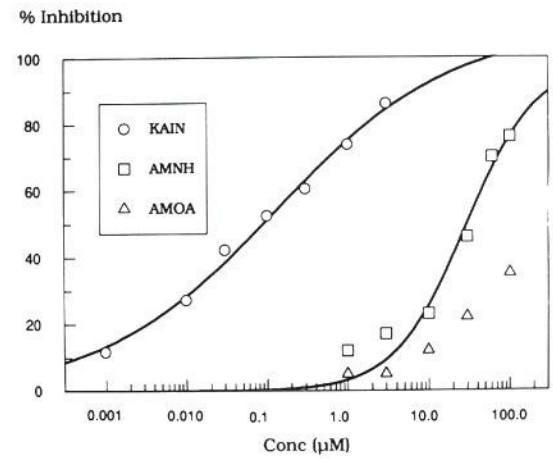


Figure 3. Inhibition of the low-affinity binding of [^{3}H]KAIN (100 mM calcium chloride added to the test system) in the presence of various concentrations of KAIN, AMOA (**7**), or AMNH (**14**). Each point is the result of at least three independent experiments. The binding assays were performed as described in the Experimental Section.

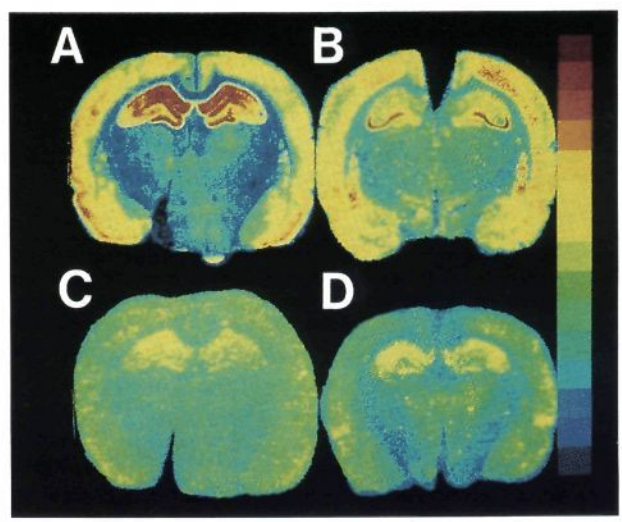


Figure 4. An illustration of the distribution of the binding sites for [^{3}H]AMPA and [^{3}H]KAIN in the presence or absence of AMOA (**7**) or AMNH (**14**) as measured by using autoradiographic techniques: (A) [^{3}H]AMPA binding at 19.5 nM; (B) [^{3}H]KAIN binding at 15 nM; (C) [^{3}H]AMPA binding at 19.5 nM with **7** (1 mM) added, (D) [^{3}H]AMPA binding at 19.5 nM with **14** (1 mM) added. For details see Experimental Section.

information derived from autoradiographic studies is more pertinent to the in vivo properties of the receptors than that obtained from receptor binding studies using isolated membrane fractions.[14,38] By use of the autoradiographic technique, the influence of AMOA (**7**) (1 mM) or AMNH (**14**) (1 mM) on [^{3}H]AMPA binding in the presence of potassium isothiocyanate (100 mM) and calcium chloride (2.5 mM) and on [^{3}H]KAIN binding in the absence of calcium chloride was determined. In agreement with earlier observations,[39,40] the binding sites for [^{3}H]AMPA and [^{3}H]KAIN were shown to be unevenly distributed in the rat brain (Figure 4). In the hippocampal formation and the cerebral cortex the concentration of binding sites are particularly high, and in the hippocampus there is a high degree of complementarity of the density of [^{3}H]-AMPA and [^{3}H]KAIN binding sites. Under the experimental conditions used, **7** displaced about 50% of [^{3}H]-AMPA binding without affecting significantly the high-

(38) Boulton, A. A.; Baker, G. B.; Hrdina, P. D., Eds. *Neuromethods. 4. Receptor Binding*; Humana Press: Clifton, New Jersey, 1986.
(39) Olsen, R. W.; Szamrej, O.; Houser, C. R. *Brain Res.* **1987**, *402*, 243.
(40) Monaghan, D. T.; Yao, D.; Cotman, C. W. *Brain Res.* **1985**, *340*, 378.

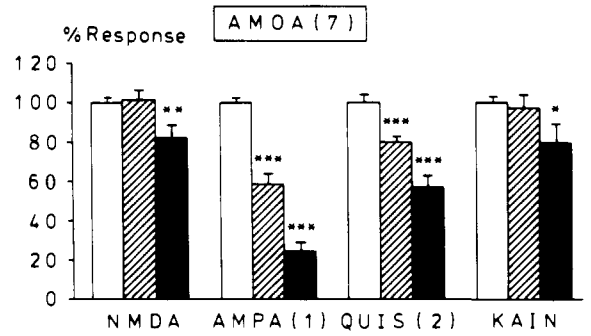


Figure 5. Effects of AMOA (7) on the neuronal excitations induced by NMDA, AMPA (1), quisqualic acid (QUIS, 2), or KAIN in a modified version[42] of the rat cortical slice preparation:[41] □, excitatory effects of NMDA (10 μM), 1 (5 μM), 2 (10 μM), or KAIN (5 μM) in the absence of antagonist; the effects of the same concentrations of agonists in the presence of 7 (250 μM, ▨, or 1 mM, ■), % response (normalized) ± SEM, $n = 3$. (*) $P < 0.05$ (paired t test), (**) $P < 0.01$, (***) $P < 0.001$.

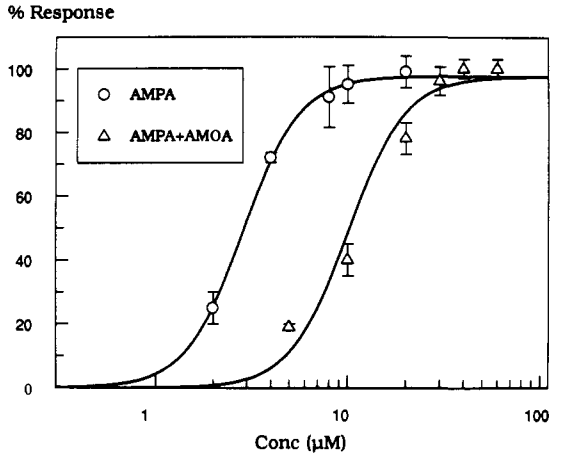


Figure 6. An illustration of the effects of AMOA (7) (800 μM) on excitations by AMPA (1) in the cortical slice preparation, % response ± SEM, $n = 3$.

affinity binding of [^{3}H]KAIN (Figure 4). 14, on the other hand, displaced about 25% of high-affinity [^{3}H]KAIN binding, whereas the binding of [^{3}H]AMPA was reduced by approximately 50% (not illustrated).

On the basis of the receptor binding studies described in the previous section, 14 seems to bind selectively to low-affinity [^{3}H]KAIN receptor sites. In light of these observations, autoradiographic studies on low-affinity [^{3}H]KAIN binding (in the presence of 100 mM calcium chloride) would be particularly relevant. So far, the technique for studies of low-affinity [^{3}H]KAIN binding has not been developed, but these aspects are at present under investigation.

In Vitro Electrophysiology. A rat cortical slice preparation[41] was used for the determination of the EAA receptor antagonist profiles of AMOA (7) and AMNH (14). As standard EAA agonists were used NMDA, AMPA (1), quisqualic acid (2), and KAIN. At a concentration of 250 μM, 7 did not significantly reduce the excitatory effects of NMDA or KAIN, whereas excitations induced by 1 or 2 were reduced by approximately 42% and 20%, respectively (Figure 5). At a higher concentration (1 mM), 7 reduced the effects of 1 by about 75% and those of 2, NMDA, and KAIN by 43%, 20%, and 20%, respectively.

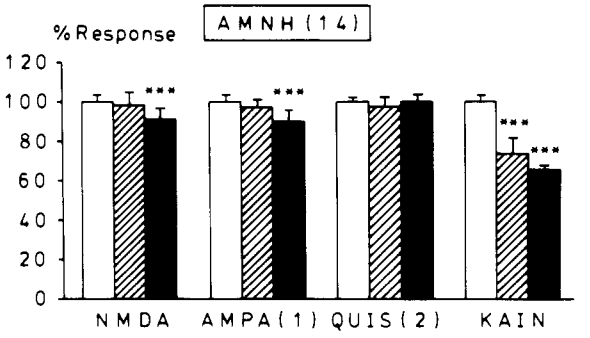


Figure 7. Effects of AMNH (14) on the neuronal excitations induced by NMDA, AMPA (1), quisqualic acid (QUIS, 2), or KAIN in a modified version[42] of the rat cortical slice preparation:[41] □ excitatory effects of NMDA (10 μM), 1 (5 μM), 2 (10 μM), or KAIN (5 μM) in the absence of antagonist; the effects of the same concentrations of agonists in the presence of 14 (100 μM, ▨, or 1 mM, ■), % response (normalized) ± SEM, $n = 6$. (*) $P < 0.05$ (paired t test), (**) $P < 0.01$, (***) $P < 0.001$.

As illustrated in Figure 6, coadministration of 7 shifted the dose–response curve for 1 to the right in a parallel fashion, indicating that 7 interacts with the AMPA receptor as a competitive antagonist.

The antagonist profile of 14 was substantially different from that observed for 7. At concentrations of 100–200 μM, 14 typically reduced the effect of KAIN by 20%, whereas excitations by NMDA, 2, or 1 were not significantly affected (Figure 7). Even at concentrations of 14 of 1 mM, the effects of KAIN were never reduced by more than 50%. At such high concentrations of 14, the effect of KAIN, 1, and NMDA were typically reduced by 35%, 15%, and 10%, respectively, whereas no significant effects on excitations by 2 were observed (Figure 7).

In all experiments where 7 was tested, virtually full recovery of the sensitivity of the cortical slice to the test agonists was observed 10–30 min after termination of the administration of 7. When the antagonist effects of 14 on NMDA-, 1-, or 2-induced excitations were determined, almost complete recovery of the slice preparations was similarly observed. However, after coadministration of 14 and KAIN to the cortical slice, termination of the application of 14 did not always result in complete recovery of the sensitivity of the cortical tissues to KAIN. Since repeated administration of KAIN alone to the brain slice for several hours did not result in similarly reduced responses to the agonist, this lack of full recovery may reflect an irreversible effect of 14 on KAIN receptors rather than neurotoxic effects of KAIN under the conditions used.

In Vivo Electrophysiology (Microelectrophoresis). The effects of AMOA (7) and AMNH (14) on cat spinal neurones were studied. Antagonism of amino acid excitation by these compounds and the NMDA antagonist 2-amino-5-phosphonopentanoic acid[43] was tested microelectrophoretically, in each case on 20–25 neurones, as described previously[44,45] by using NMDA, 2, 1, and KAIN as reference excitants (Figure 8). In the assessment of the relative potencies of antagonists the addition of sodium chloride (150 mM) to dilute solutions (20 or 50 mM) of antagonists was taken into account (see Experimental Section). In agreement with earlier findings, 2-amino-5-phosphonopentanoic acid was an effective and highly se-

(41) Harrison, N. L.; Simmonds, M. A. *Br. J. Pharmacol.* **1985**, *84*, 381.
(42) Wheatley, P. L. *Br. J. Pharmacol.* **1986**, *87*, 159P.
(43) Watkins, J. C.; Olverman, H. J. *Trends Neurosci.* **1987**, *10*, 272.
(44) Lodge, D.; Headley, P. M.; Curtis, D. R. *Brain Res.* **1978**, *153*, 603.
(45) Curtis, D. R.; Duggan, A. W.; Felix, D.; Johnston, G. A. R. *Brain Res.* **1971**, *32*, 69.

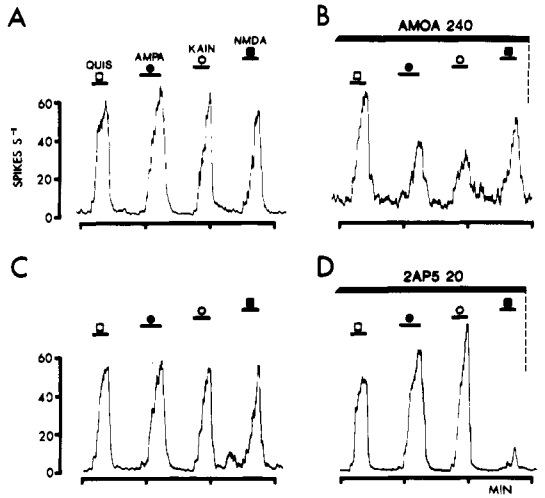


Figure 8. (A,B) Effects of AMOA (7) on the excitation of a spontaneously firing interneurone by quisqualic acid (QUIS, 2), (70 nA), AMPA (1) (20 nA), KAIN (80 nA), and NMDA (50 nA) ejected with the indicated currents (nanoamperes) and for the times shown by the horizontal bars and symbols: (A) before and (B) during 7 (240 nA), starting and ceasing indicated. (C,D) Effects of 2-amino-5-phosphonopentanoic acid (2AP5) on the excitation of a spontaneously firing interneurone by 2 (70 nA), 1 (20 nA), KAIN (80 nA), and NMDA (50 nA): (C) before and (D) during 2AP5 (20 nA). Ordinates are firing rate in spikes per second. Abscissae are time in minutes.

lective antagonist of NMDA-induced excitation.[1,43]

Although the absolute and relative sensitivity of neuronal excitations produced by the standard agonists to 2-amino-5-aminopentanoic acid, 7, and 14 on different cells varied, excitations by NMDA were never significantly reduced by 7 or 14. Similarly, on the majority of cells studied the sensitivity of excitations by 2 to 7 or 14 was markedly lower than excitations produced by AMPA. On a number of cells the amounts of 7 or 14 sufficient to reduce the effects of 1 by 50–70% only marginally affected excitations by 2 as exemplified in Figure 8. On the other hand, the degree of selectivity demonstrated for 7 and 14 as AMPA and KAIN receptor antagonists, respectively, using the cortical slice model was only seen on a few cat spinal neurones. On a majority of these neurones, excitations by 1 or KAIN were reduced to approximately the same level. On some spinal neurones, the effects of KAIN were more sensitive to these antagonists, in particular to 14, than were those produced by 1 (not illustrated).

Neuroprotection. The abilities of AMOA (7) and AMNH (14) to protect rat striatal neurones against the neurotoxic effects of 1, KAIN, and the NMDA agonist quinolinic acid were tested and compared with the neuroprotective effects of the NMDA antagonist CPP.[1,43,46] As expected, intrastriatal injections of 1, quinolinic acid, or KAIN produced significant declines in the activities of choline acetyltransferase and glutamic acid decarboxylase relative to the contralateral striatum (Table II). Coinjection of CPP, but not 7 or 14, effectively prevented quinolinic acid induced neuronal loss. Like CPP, 14 was ineffective in preventing the neurotoxic effects of 1, whereas 7 showed weak neuroprotective effects under these conditions. On the other hand, 7 completely attenuated striatal cell damage in the presence of KAIN. Likewise, coinjected 14 inhibited the neurotoxic effects of KAIN, though less effectively (Table II).

Table II. Neuroprotective Effects of Some Excitatory Amino Acid Antagonists

agonist[a]	antagonist[a]	number of animals	percent enzyme activity[b] choline acetyltransferase	percent enzyme activity[b] glutamic acid decarboxylase
1		14	53 ± 6	40 ± 12
1	+ 7	14	68 ± 7*	62 ± 9*
1	+ 14	9	53 ± 10	39 ± 7
1	+ CPP	7	55 ± 8	50 ± 13
QUIN		14	61 ± 8	53 ± 8
QUIN	+ 7	3	52 ± 2	64 ± 11
QUIN	+ 14	5	50 ± 12	49 ± 4
QUIN	+ CPP	5	98 ± 3***	99 ± 4***
KAIN		7	34 ± 5	44 ± 8
KAIN	+ 7	10	90 ± 7***	102 ± 10***
KAIN	+ 14	10	87 ± 10***	68 ± 4**

[a] Solutions (1 μL) containing KAIN (10 nmol), quinolinic acid (QUIN) (300 nmol), or AMPA (1) (100 nmol) with or without the test compounds, CPP (150 nmol), AMOA (7) (150 nmol), or AMNH (14) (150 nM) were infused into the corpus striatum of rats. [b] Following a 3-day recovery period, animals were sacrificed and the percent remaining activities of the marker enzymes choline acetyltransferase[47] and glutamic acid decarboxylase[48] determined. For further details see Experimental Section. (*) $P < 0.05$ compared to agonist alone (Student's t test), (**) $P < 0.01$, (***) $P < 0.005$.

Discussion. During the past few years the pharmacological characteristics of the recognition and modulatory sites of the NMDA receptor complex have been mapped out in some detail.[7,12,14] These studies have been facilitated by the availability of a series of highly selective competitive NMDA receptor antagonists, which typically are phosphono amino acids with chain lengths longer than those of NMDA and Glu.[1,7,14,43]

Within the class of non-NMDA receptors the AMPA (1) and KAIN receptors have, so far, been most extensively studied.[5,7,14,15] There is evidence to suggest that AMPA receptors exist in two interconvertible states, the equilibrium of which is affected by isothiocyanate ions.[5,23] The KAIN receptors exist in two states showing different agonist affinities, but in the presence of millimolar concentrations of calcium ions only low-affinity KAIN receptor sites, which probably reflect physiologically relevant KAIN receptors,[5] can be detected. A number of potent non-NMDA receptor antagonists have been developed, notably different quinoxalinediones, including CNQX and NBQX, but none of these compounds selectively block excitatory effects induced by 1 or KAIN.[5,17,18]

In this paper we describe two novel non-NMDA receptor antagonists, AMOA (7) and AMNH (14). There is a conspicuous lack of structural similarity between the quinoxalinediones and acidic amino acids, whereas the isoxazoles 7 and 14 are acidic amino acids having carbon backbones longer than those of 1 or KAIN (Figure 1).

In the cortical slice preparation, 7 blocks AMPA-induced excitation competitively (Figure 6) with some selectivity, being less potent as an antagonist of excitations by quisqualic acid (2) and showing only weak effects on excitations mediated by NMDA or KAIN receptors (Figure 5). In this cortical tissue 14 does, however, consistently antagonize excitations by KAIN more effectively than those induced by 1 or 2 (Figure 7). This effect seems particularly interesting, since 14, in contrast to 7, appears to bind to low-affinity [^{3}H]KAIN sites, supporting the view that, at least in cortical tissues, low-affinity KAIN binding sites may be physiologically relevant.[17,24] Compound 14 seems to antagonize only a fraction of the KAIN response, since the effects of KAIN never were blocked by more than 50% (Figure 7). It has been proposed that AMPA recep-

(46) Murphy, D. E.; Schneider, J.; Boehm, C.; Lehmann, J.; Williams, M. *J. Pharmacol. Exp. Ther.* **1987**, *240*, 778.
(47) Bull, G.; Oderfeld-Nowak, B. *J. Neurochem.* **1971**, *19*, 935.
(48) Wilson, S. H.; Schrier, B. K.; Fader, J. L.; Thompson, E.; Rosenberg, R. N.; Blume, A. J.; Nirenberg, M. W. *J. Biol. Chem.* **1972**, *247*, 3155.

tors largely mediate the neuronal depolarization by 1 as well as 2 and KAIN.[5,14,17,25] This may be the case in the mammalian spinal cord, where both 7 and 14 show very similar effects on 1- and KAIN-induced excitations, although the effects on excitations by 2 were consistently weaker (Figure 7). However, in light of the very different antagonist effects of 7 and 14 in the cortical slice preparation, the depolarizations by 1 and KAIN in the brain are likely to be mediated by separate but, perhaps, associated receptor mechanisms as proposed on the basis of analyses of whole-cell[49] and single-channel[50] currents. Interestingly, recent findings suggest the existence of qualitative and quantitative differences between spinal and cortical AMPA receptors.[21]

In the cat spinal cord (Figure 8) and in the rat cortical slice (Figure 5) excitation by 2 was markedly less sensitive to antagonism by 7 than excitation induced by 1, and in the latter system, 14 did not significantly reduce the effect of 2 (Figure 7). Compound 2 is at least as potent as 1 as an inhibitor of [^{3}H]AMPA binding[23,36] and as a neuronal excitant,[7] but whereas 1 is a highly selective AMPA receptor agonist, 2 is capable of activating metabotropic receptors[51,52] as well as AMPA receptors. The results of the present studies seem to indicate that synaptic effects by 2 are not primarily mediated by AMPA receptors and only to a very limited extent by low-affinity KAIN receptors. It is possible that metabotropic receptors play an important role in mediating the effects of QUIS.

It is noteworthy that 7 shows very weak and 14 virtually no effect on the neurotoxic actions of 1 in the rat corpus striatum (Table II). On the other hand, 14 and, even more pronounced, 7 effectively protected striatal neurones against KAIN-induced neurotoxicity. These results compared with the pharmacological data discussed above seem to indicate that the neurotoxic effects of 1 and KAIN are not mediated by the same receptor mechanisms, and furthermore, the mechanisms underlying depolarization and neurotoxicity are not identical. The very weak effects of 7 and 14 on the neurotoxic effects of AMPA (1) may suggest that these AMPA analogues actually are acting as low-efficacy partial agonists at AMPA receptors. Electrophysiological studies in progress may shed light on these mechanistic aspects.

Studies along different lines have been initiated in order to shed some more light on the precise mechanism of action of 7 and 14, which represent a novel class of non-NMDA EAA antagonists. These new pieces for the EAA receptor "jig-saw puzzle" may be useful tools for further studies of the complex mechanisms involved in EAA neurotransmission and -toxicity.

Experimental Section

Chemistry. General Procedures. Melting points are corrected and were determined in capillary tubes. Elemental analyses were performed by Mr. P. Hansen, Chemical Laboratory II, University of Copenhagen or by Mr. G. Cornali, Microanalytical Laboratory, Leo Pharmaceutical Products, Copenhagen. IR spectra, obtained on a Perkin-Elmer 781 infrared spectrophotometer, were recorded in KBr pellets. ^{1}H NMR spectra were recorded on a Varian EM 360L spectrometer or a JEOL FX 90Q instrument. TMS was used as an internal standard except for the compounds dissolved in D_2O, where sodium 3-(trimethylsilyl)propanesulfonate was used. Thin-layer chromatography (TLC) and gravity column chromatography (CC) were performed by using silica gel F_{254} plates (Merck) and silica gel (Woelm, 0.063-0.200 mm), respectively. Compounds containing the 3-isoxazolyl unit were visualized on TLC plates by using UV light and a $FeCl_3$ spraying reagent (yellow color). Compounds containing amino groups were visualized by using a ninhydrin spraying reagent, and all compounds under study were detected on TLC plates by using a $KMnO_4$ spraying reagent. All evaporations were performed at ca. 15 mmHg with use of a rotary evaporator.

Methyl 2-Acetamido-2-(methoxycarbonyl)-3-(3-hydroxy-5-methylisoxazol-4-yl)propionate (4). A mixture of 3[15] (9.1 g, 29 mmol) and a solution of hydrogen bromide in glacial acetic acid (100 mL, 33%) was left at room temperature for 20 h. The solution was concentrated to 15 mL, and upon addition of ether (15 mL) followed by light petroleum (15 mL), 8.10 g (93%) of 4 crystallized, mp 220–221 °C. ^{1}H NMR (90 MHz, $CDCl_3$): δ 8.25 (2 H, s), 3.66 (6 H, s), 3.12 (2 H, s), 2.10 (3 H, s), 1.88 (3 H, s). Anal. ($C_{12}H_{16}N_2O_7$) C, H, N.

Methyl 2-Acetamido-2-(methoxycarbonyl)-3-[3-[(ethoxycarbonyl)methoxy]-5-methylisoxazol-4-yl]propionate (6). A suspension of 4 (3.8 g, 12.7 mmol) and finely powdered potassium carbonate (4.4 g, 31.8 mmol) in dry acetone (75 mL) was stirred at 50 °C for 90 min. Upon addition of ethyl chloroacetate (5.0 g, 38.1 mmol) the mixture was stirred for 20 h at 50 °C. The reaction mixture was filtered and the evaporated filtrate subjected to CC [silica gel, 200 g; eluents, toluene containing ethyl acetate (8–20%)] to give 6 (1.36 g, 28%), mp 93.0–94.0 °C (ethyl acetate–light petroleum). ^{1}H NMR (90 MHz, CCl_4): δ 7.22 (1 H, s), 4.68 (2 H, s), 4.22 (2 H, q, J = 7 Hz), 3.70 (6 H, s), 3.27 (2 H, s), 2.20 (3 H, s), 1.91 (3 H, s), 1.30 (3 H, t, J = 7 Hz). Anal. ($C_{16}H_{22}N_2O_9$) C, H, N.

(*RS*)-2-Amino-3-[3-(carboxymethoxy)-5-methylisoxazol-4-yl]propionic Acid Sesquihydrate (AMOA, 1.5 H_2O) (7). A suspension of 6 (1.4 g, 3.5 mmol) in aqueous hydrochloric acid (30 mL, 2 M) was refluxed for 10 h. The solution was then evaporated and dissolved in water (3 mL). Upon filtration ethanol (3 mL) was added and pH of the solution was adjusted to 5 by addition of triethylamine (TEA). The precipitated crystalline product was recrystallized (H_2O) to give 7 (377 mg, 40%), mp 230 °C dec. ^{1}H NMR (90 MHz, D_2O): δ 4.74 (2 H, s), 4.05 (1 H, t, J = 7 Hz), 2.95 (2 H, d, J = 7 Hz), 2.24 (3 H, s). Anal. ($C_9H_{12}N_2O_6 \cdot 1.5\ H_2O$) C, H, N.

4-(Chloromethyl)-2-[(3-methoxy-5-methylisoxazol-4-yl)-methyl]-5-methylisoxazolin-3-one (11) and 4-(Chloromethyl)-5-methyl-2-[5-methyl-2-[(3-methoxy-5-methylisoxazol-4-yl)methyl]-3-oxoisoxazolin-4-yl]isoxazolin-3-one (9). Compound 8[15] (1.0 g, 6.2 mmol) was heated at 180 °C for 3 h. The crystalline reaction mixture was subjected to CC [silica gel, 35 g; eluents, toluene–ethyl acetate–ethanol (4:1:1)] to give unreacted 8 (190 mg). 11 (101 mg; 12%) was obtained, mp 82.0–84.0 °C (ethyl acetate–light petroleum). ^{1}H NMR (60 MHz, $CDCl_3$): δ 4.70 (2 H, s), 4.32 (2 H, s), 3.99 (3 H, s), 2.38 (3 H, s), 2.32 (3 H, s). Anal. ($C_{11}H_{13}N_2O_4Cl$) C, H, N, Cl. Compound 9 (36 mg; 3%) was isolated, mp 114.0–116.0 °C (ethyl acetate–light petroleum). ^{1}H NMR (90 MHz, $CDCl_3$): δ 4.67 (4 H, s), 4.28 (2 H, s), 3.97 (3 H, s), 2.36 (3 H, s), 2.29 (6 H, s). Anal. ($C_{16}H_{18}N_3O_6Cl$) C, H, N, Cl; C: calcd 50.05; found, 49.23.

Methyl 2-Acetamido-2-(methoxycarbonyl)-3-[2-[(3-methoxy-5-methylisoxazol-4-yl)methyl]-5-methyl-3-oxoisoxazolin-4-yl]propionate (12). To a solution of sodium methoxide prepared from sodium (25 mg, 1.1 mmol) and methanol (4 mL) was added dimethyl acetamidomalonate (AAMM) (208 mg, 1.1 mmol) and 11 (273 mg, 1.0 mmol). The mixture was refluxed for 3 h and then evaporated, and upon addition of water (5 mL), the residue was extracted with chloroform (3 × 10 mL). The combined organic phases were washed with iced aqueous sodium hydroxide (10 mL, 1 M), dried ($MgSO_4$), and evaporated. Recrystallization (methanol–ethyl acetate–light petroleum) of the residue gave 12 (197 mg, 46%), mp 164.0–166.0 °C. ^{1}H NMR (90 MHz, $CDCl_3$): δ 7.17 (1 H, br s), 4.63 (2 H, s), 3.98 (3 H, s), 3.80 (6 H, s), 3.23 (2 H, s), 2.35 (3 H, s), 2.09 (3 H, s), 2.00 (3 H, s). Anal. ($C_{18}H_{23}N_3O_9$) C, H, N.

(*RS*)-2-Amino-3-[2-[(3-methoxy-5-methylisoxazol-4-yl)-methyl]-5-methyl-3-oxoisoxazolin-4-yl]propionic Acid Hydrochloride (13). A mixture of 12 (850 mg, 2 mmol) and aqueous hydrochloric acid (30 mL, 1 M) was refluxed for 4 h. The solution was evaporated and the residue recrystallized (methanol–ether) to give 13 (0.64 g, 92%), mp 208 °C dec. ^{1}H NMR (90 MHz, D_2O):

(49) Perouansky, M.; Grantyn, R. *J. Neurosci.* **1989**, *9*, 70.
(50) Ascher, P.; Novak, L. *J. Physiol. (London)* **1988**, *399*, 227.
(51) Recasens, M.; Guiramand, J.; Nourigat, A.; Sassetti, I.; Devilliers, G. *Neurochem. Int.* **1988**, *13*, 463.
(52) Murphy, S. N.; Miller, R. J. *Mol. Pharmacol.* **1989**, *35*, 671.

δ 4.77 (2 H, s), 4.13 (1 H, t, J = 6 Hz), 3.86 (3 H, s), 2.85 (2 H, d, J = 6 Hz), 2.30 (3 H, s), 2.16 (3 H, s). Anal. ($C_{13}H_{18}N_3O_6Cl$) C, H, N, Cl.

(*RS*)-2-Amino-3-[2-[(3-hydroxy-5-methylisoxazol-4-yl)-methyl]-5-methyl-3-oxoisoxazolin-4-yl]propionic Acid (AMNH) (14). A solution of 13 (0.64 g, 1.84 mmol) in a solution of hydrogen bromide in glacial acetic acid (15 mL, 33%) was left at 25 °C for 24 h. The solution was evaporated and a solution of the residue in water (2 mL) was transferred to a column containing an ion-exchange resin [Amberlite IRA-400 (OH); 100 mL]. The column was eluted with acetic acid (1 M) to give 14 (242 mg, 44%) after recrystallization (H_2O), mp 235 °C dec. ^1H NMR (90 MHz, D_2O): δ 4.76 (2 H, s), 3.89 (1 H, t, J = 6 Hz), 2.78 (2 H, d, J = 6 Hz), 2.29 (3 H, s), 2.16 (3 H, s). Anal. ($C_{12}H_{15}N_3O_6$) C, H, N.

Stability of AMOA (7) and AMNH (14). Solutions of AMOA (7) (2.5 mg) and AMNH (14) (2.3 mg) in phosphate buffer (0.25 mL, 50 mM, pH 7.4) adjusted to an ionic strength of 0.5 by addition of potassium chloride were left at 25 °C for 14 days. The homogeneity of each solution was tested every day by TLC [eluent, 1-butanol–glacial acetic acid–water (4:1:1)]. All of the analyses revealed only one spot on the TLC plates for each of the solutions, showing R_f values of 0.11 (AMOA) and 0.19 (AMNH).

Receptor Binding Assays. The membrane preparation used in the [^{3}H]AMPA, [^{3}H]KAIN, [^{3}H]CPP, [^{3}H]MK-801, and [^{3}H]glycine binding assays was prepared according to the method of Ransom and Stec.[53]

The [^{3}H]AMPA binding assay was performed by following a published procedure.[23]

High-affinity [^{3}H]KAIN binding was performed as described[24] with the following modifications: (1) the concentration of [^{3}H]KAIN was 5 nM rather than 1 nM; (2) 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). Low-affinity [^{3}H]KAIN binding was studied by using the assay conditions described by Braitman and Coyle[24] in the presence of 30 nM [^{3}H]KAIN and 100 mM calcium chloride. Furthermore, the assays were terminated by centrifugation at 30000g for 5 min. The resulting pellets were superficially rinsed in ice-cold 50 mM Tris-HCl buffer (2 × 5 mL, pH 7.1) rather than with water.

[^{3}H]CPP binding was studied essentially by following a published procedure,[46] but the centrifugation step was replaced by a filtration procedure using Whatman GF/B filters.

[^{3}H]MK-801 binding assays were performed essentially as described earlier,[54] 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.

The [^{3}H]glycine binding assays were accomplished as described by Ransom and Stec.[53]

Studies on the effects on the binding of [^{3}H]GABA to $GABA_A$ or $GABA_B$ receptor sites were performed as described previously.[55]

Receptor Autoradiography. Frozen coronal sections (20 μm) from control male Wistar rats were preincubated for 10 min in 50 mM Tris–acetate buffer following incubation for 30 min at 20 °C in 50 mM Tris–acetate buffer with 100 mM KSCN. [^{3}H]-AMPA was added in the following concentrations: 4.75, 9.5, 19, 38, or 76 nM. In parallel series of incubations 1 mM 14 or 1 mM 7 were added to the vials. Nonspecific binding was assayed at 38 nM [^{3}H]AMPA with 1 mM Glu added. Corrections for nonspecific binding were made, assuming a linear relationship. Sections were washed for 3 × 30 s in Tris–acetate buffer followed by a short wash (1 s) in 5% glutaraldehyde in acetone. The sections and 20 calibrated [^{3}H]methylmethacrylate standards were exposed to a [^{3}H]hyperfilm for 45 days for the two higher ligand concentrations and 90 days for the three lowest concentrations before being developed. [^{3}H]KAIN binding was assayed after 10-min incubation in Tris–citrate buffer followed by 60-min incubation in Tris–citrate with [^{3}H]KAIN added in the following concentrations: 7, 15, 30, 60, or 120 nM. A parallel series of vials were added 1 mM 7 or 1 mM 14. Nonspecific binding was assayed at 60 nM [^{3}H]KAIN with 1 mM Glu added. Sections were washed in 30 mM Tris-HCl buffer for 3 × 30 sec followed by 1 s wash in 5% glutaraldehyde in acetone. Exposure times for sections and high and low standards were 45 and 90 days, respectively. Regional analyses of binding were made with a Leitz TAS computerized video-densitometer. Pseudocolor-coded images (256 × 256 pixels) were made by using a PDP 11/73 computer with a Seiko display unit.

Electrophysiology. A modified version[42] of the cortical slice preparation[41] was used for studies of the effects of the test compounds on cortical tissue in vitro.

Microelectrophoretic studies were performed on lumbar dorsal horn interneurones or Renshaw cells of cats anaesthetized with pentobarbitone sodium (35 mg kg^{-1} intraperitoneally initially, supplemented intraveneously when required). Extracellular action potentials were recorded by means of the central barrel of seven-barrel micropipets, which contained 3.6 M sodium chloride. The compounds were administered electrophoretically from the outer barrels of the micropipets,[45] which contained aqueous solutions of 2 (5 mM in 150 mM sodium chloride, pH 7.5), 1 (100 mM, pH 7.0), KAIN (5 mM in 150 mM sodium chloride, pH 7.0), NMDA (50 mM in 150 mM sodium chloride, pH 7.0), 7 (20 mM in 150 mM sodium chloride, pH 7.0), 14 (20 mM in 150 mM sodium chloride, pH 7.0), and 2-amino-5-phosphonopentanoic acid (50 mM in 150 mM sodium chloride, pH 7.0). The excitatory amino acids were administered for periods of time sufficient to obtain maximal effects of the particular rate of ejection.

Neuroprotection. Rats were anaesthetized with Chloropent (4 mL per kg intraperitoneally) and placed in a David–Kopf stereotaxic apparatus. A 0.3-mm stainless steel cannula was lowered into the corpus striatum through a small bore hole in the calvarium; coordinates for the injections were A 0.8, L 3.0, V 4.4 (midline, bregma, and dura = 0.0). KAIN was dissolved in saline (0.9% w/v); all other drugs were dissolved in distilled water. Solutions were adjusted to neutral pH by using NaOH, and 1 μL of solutions containing KAIN (10 nmol), quinolinic acid (300 nmol), or 1 (100 nmol) with or without the test compounds, CPP (150 nmol), AMOA (7) (150 nmol), or AMNH (14) (150 nmol) was infused over a period of 60 s. Following a delay of 60 s, the cannula was slowly retracted and the scalp apposed with autoclips. Following a 3-day recovery period, animals were sacrificed by decapitation. The corpus striatum was dissected, weighed and frozen (−80 °C) until assay. For measurements of enzyme activities, striatal tissue was homogenized in 50 mM Tris-HCl buffer (pH 7.4, 23 °C) containing Triton X-100 (0.2% w/v). After centrifugation at 10000g for 10 min (23 °C) a portion of the supernatant was assayed for choline acetyltransferase[47] and glutamic acid decarboxylase[48] activities by following published procedures. The values shown represent the per cent remaining activity of each enzyme relative to the non-lesioned striatal tissues after injections of antagonists and/or agonists.

Acknowledgment. This work was supported by grants from the Danish Medical and Technical Research Councils, The Australian National University, and the Lundbeck Foundation. The secretarial and technical assistance, respectively, of Mrs. B. Hare and Mrs. P. Searle are gratefully acknowledged.

Registry No. 3, 130146-20-2; 4, 130146-21-3; 6, 130146-22-4; 7, 130146-18-8; 8, 75989-22-9; 9, 130146-23-5; 11, 130146-24-6; 12, 130146-25-7; 13, 130146-26-8; 14, 130146-19-9; AAMM, 60187-67-9; $ClCH_2COOEt$, 105-39-5.

(53) Ransom, R. W.; Stec, N. L. *J. Neurochem.* **1988**, *51*, 830.
(54) Foster, A. C.; Wong, E. H. F. *Br. J. Pharmacol.* **1987**, *91*, 403.
(55) Falch, E.; Hedegaard, A.; Nielsen, L.; Jensen, B. R.; Hjeds, H.; Krogsgaard-Larsen, P. *J. Neurochem.* **1986**, *47*, 898.