(3*SR*,4a*RS*,6*RS*,8a*RS*)-6-[2-(1*H*-Tetrazol-5-yl)ethyl]decahydroisoquinoline-3-carboxylic Acid: A Structurally Novel, Systemically Active, Competitive AMPA Receptor Antagonist

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Received April 23, 1993

The recent discovery of **1** (DNQX, Chart I) and **2** (CNQX, Chart I) as competitive antagonists[1] of neurotransmission mediated through the AMPA [2-amino-3-(5-methyl-3-hydroxyisoxazol-4-yl)propanoic acid, **3**, Chart I) subclass of excitatory amino acid (EAA) receptors[2] has been instrumental in providing a greater understanding of the pharmacology of this class of compounds. Their utility as therapeutic agents was limited, however, by their lack of activity following systemic administration in animals. Further structure–activity studies led to the discovery of **4**[3] (NBQX, Chart I), an important novel agent which has enhanced understanding of the therapeutic potential of AMPA antagonists. For example, **4** has anticonvulsant[4] and neuroprotective[5] properties and is effective in animal models of global[3,6] and focal cerebral ischemia.[7]

The 2,3-benzodiazepine **5** (GYKI 52466, Chart I) was recently identified as a noncompetitive AMPA antagonist.[8] This compound is structurally distinct from and acts at a different site than **4** and is neuroprotective in animal models of ischemia.[6,9] The acidic amino acid **6** (AMOA, Chart I) was also shown to be a competitive AMPA antagonist with neuroprotective properties.[10] The data accumulated from studies with competitive and noncompetitive AMPA antagonists strengthens the hypothesis for the potential utility of an AMPA antagonist in the treatment of stroke and other conditions of acute neuronal degeneration that may involve glutamate neurotoxicity. One liability of **4** and **5**, however, is their limited solubility in aqueous solution, which may hamper their use as parenterally administered drugs; and **6** is not active following systemic administration. Therefore, the identification of potent, systemically active, water-soluble AMPA antagonists remains an important goal.

We recently described that **7** [(3*SR*,4a*RS*,6*SR*,8a*RS*)-6-(phosphonomethyl)-1,2,3,4,4a,5,6,7,8,8a-decahydroisoquinoline-3-carboxylic acid, LY274614] and **8** [(3*SR*,4a*RS*,6*SR*,8a*RS*)-6-[2-(1*H*-tetrazol-5-yl)methyl]-1,2,3,4,4a,5,6,7,8,8a-decahydroisoquinoline-3-carboxylic acid, LY233536] were potent and selective NMDA antagonists.[11,12] In the course of our structure–activity studies on this series of 6-substituted decahydroisoquinoline-3-carboxylic acids, we discovered that the tetrazolylethyl analog **9** [(3*SR*,4a*RS*,6*RS*,8a*RS*)-6-[2-(1*H*-tetrazol-5-yl)-ethyl]-1,2,3,4,4a,5,6,7,8,8a-decahydroisoquinoline-3-carboxylic acid, LY215490] was an AMPA receptor antagonist. In this communication we describe the synthesis and pharmacological activity of **9**, a potent, systemically active, competitive AMPA antagonist.

Chemistry. Scheme I shows the preparation of amino acid (±)-**9**. Stereoselective hydroboration of the olefin **11**[13] (BH_3·Me_2S, THF, 0 °C; EtOH, 3 N NaOH, 30% H_2O_2, 0 °C), obtained from the known racemic ketone **10**[14] by a Wittig olefination ($Ph_3PCH_3^+Br^-$, $NaN(SiMe_3)_2$, THF, 0 °C), gave the hydroxymethyl compound **12**[13] (>10:1 by ^1H NMR).[14] Subsequent oxidation of **12** afforded the aldehyde **13**[13] (oxalyl chloride, DMSO, CH_2Cl_2, Et_3N, −78 °C), and condensation with the sodium salt of diethyl phosphonoacetonitrile gave the α,β-unsaturated nitrile **14**[13] (NaH, $(EtO)_2P(O)CH_2CN$, THF, room temperature). Under these Horner–Emmons reaction conditions, there was no epimerization of the aldehyde at C-6. Catalytic hydrogenation of the double bond in **14** to the saturated nitrile **15a** proved troublesome, often giving significant amounts of overreduction to the primary amine **16**. We avoided this problem by performing the reduction with magnesium in methanol,[15] which afforded a mixture of the ethyl and methyl esters **15a**[13] and **15b**.[13] Conversion of this mixture of cyano-substituted esters to the tetrazole with azidotri-*n*-butylstannane (neat, 80 °C), exhaustive hydrolysis (6 N HCl, 90 °C), removal of tin-containing byproducts by extraction with ether and then adjusting the pH to 5 gave the desired amino acid (±)-**9**.[13]

To obtain the (−)- and (+)-enantiomers of **9**, the above sequence was carried out starting with (−)- and (+)-**10**,[14] respectively. The amino acids were isolated by cation exchange chromatography on Dowex 50-X8 (100–200 mesh), eluting with 10% pyridine/water.

Pharmacology. The amino acid **9** was evaluated for affinity at various EAA receptor subclasses using the selective radioligands [^{3}H]AMPA,[16] [^{3}H]CGS 19755[17] (for NMDA receptors), and [^{3}H]kainic acid.[18] Antagonist activity and potency was determined in a cortical slice preparation[19] versis AMPA (40 μM), NMDA (40 μM), and kainic acid (10 μM). The amino acid **9** showed selective affinity for the AMPA receptor, with an IC_{50} (± SEM) of 4.81 ± 1.23 μM versus [^{3}H]AMPA binding. Compound **9** had lower affinity for the NMDA and kainic acid receptors, with IC_{50}s of 26.4 ± 1.9 and 247 ± 8 μM versus [^{3}H]CGS 19755 and [^{3}H]kainic acid binding, respectively. Amino acid **9** antagonized AMPA-induced depolarizations in a cortical slice assay, with an IC_{50} of 6.0 ± 1.0 μM. Amino acid **9** also inhibited kainic acid- and NMDA-induced depolarizations, but was 5- and 10-fold less potent, with IC_{50}s of 31.7 ± 4.4 and 61 ± 3 μM, respectively. Consistent with competitive antagonism of AMPA receptors in this assay, **9** produced rightward shifts in the AMPA concentration–effect curves with a pA_2 value of 6.37 ± 0.02. Amino acid **9** did not displace [^{3}H]glycine binding (at doses up to 100 μM), and its NMDA antagonist activity measured in the cortical slice assay was not overcome by glycine (100 μM or 10 mM), indicating no affinity for glycine receptors.

This novel AMPA antagonist was evaluated for its ability to block rigidity in mice induced by a 30 mg/kg intravenous dose of **17**[20] (ATPA; Chart I). Compound **17** is a selective agonist[21] that is less potent than AMPA but has better activity following systemic administration. This is the

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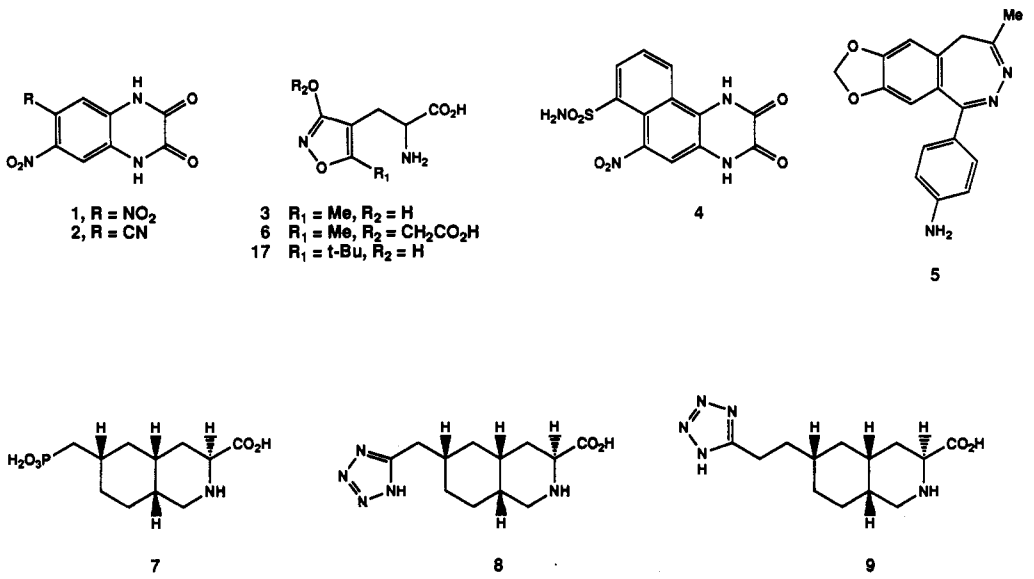
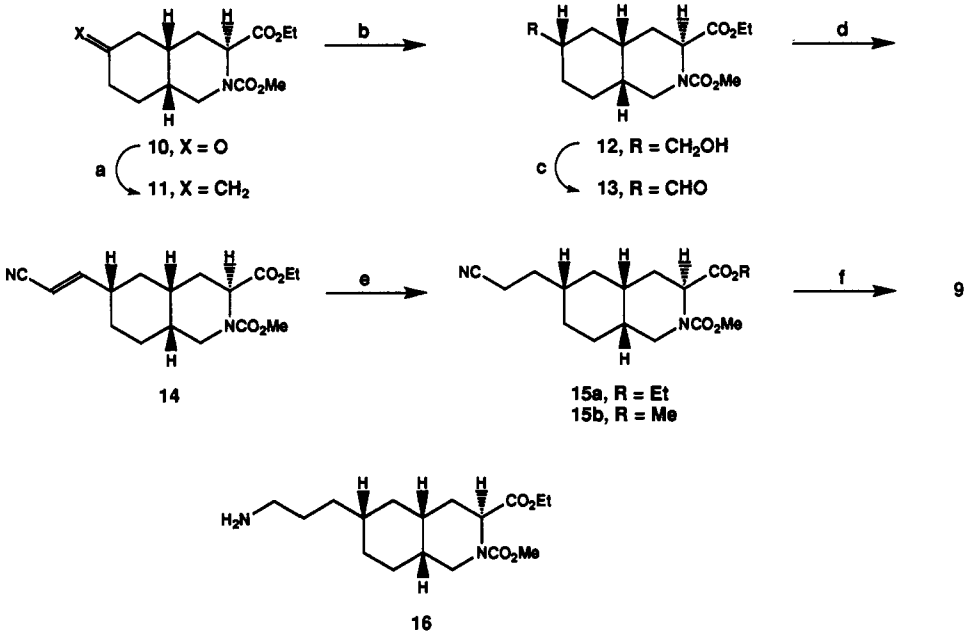
0022-2623/93/1836-2046$04.00/0

Chart I



Scheme I[a]



[a] (a) $(Ph_3PCH_3)^+Br^-$, $Na(NSiMe_3)_2$, THF 0 °C; **10**, THF, 0 °C, 95%. (b) $BH_3{\cdot}SMe_2$, THF, 0 °C to room temperature; ethanol, 3 N NaOH, 30% H_2O_2, 0 °C to room temperature, 93%. (c) DMSO, oxalyl chloride, Et_3N, CH_2Cl_2, −78 °C, 100%. (d) $(EtO)_2P(O)CH_2CN$, NaH, THF, 0 °C to room temperature, 95%. (e) Mg, MeOH, room temperature to 0 °C, 74%. (f) *n*-Bu_3SnN_3, 80 °C; 6N HCl, 90 °C; ether extraction; adjust pH to 5, 55%.

first and only assay available for evaluation of the behavioral effects of a selective AMPA agonist in vivo. Amino acid **9** showed a dose-dependent block of the effects of **17** in mice following intraperitoneal (ip) administration 30 min prior to testing, with an ED_{50} = 3.6 mg/kg [95% confidence interval (CI) = 1.7–6.3] (Figure 1). We evaluated the anticonvulsant effects of **9** using the maximal electroshock induced seizure assay in mice.[22] Amino acid **9** blocked these seizures with an ED_{50} = 9.0 mg/kg (95% CI = 6.8–11.8) (ip, 30 min prior to testing) (Figure 1). At doses up to 320 mg/kg (ip, 30 min prior to testing), **9** was ineffective in blocking NMDA-induced lethality in mice.[23] As a measure of neurological impairment of this AMPA antagonist, we evaluated **9** in a horizontal screen assay.[24] We observed that **9** was disruptive on the horizontal screen with an ED_{50} = 19.6 mg/kg (95% CI = 15.3–25.6) (ip, 30 min prior to testing). The ED_{50} dose for disruption on the horizontal screen is 5.4 and 2.2 times greater than the ED_{50} values for blocking ATPA-induced rigidity and MES-induced seizures in mice, respectively. This may indicate the potential for a good separation between therapeutic effects and side effects for this compound.

Resolution of the amino acid **9** afforded (−)-**9** (LY293558) and (+)-**9**. The isomer (−)-**9** displaced the binding of [^{3}H]-AMPA and [^{3}H]NMDA with IC_{50}s of 1.35 ± 0.13 and 12.1 ± 2.0 μM, whereas the isomer (+)-**9** was inactive (no significant inhibition of [^{3}H]AMPA or [^{3}H]NMDA binding at doses up to 100 μM). In the cortical slice assay (−)-**9** selectively reduced depolarizations due to 40 μM AMPA with an IC_{50} of 1.78 ± 0.23 μM. The absolute stereochemistry of the active isomer (−)-**9** (3*S*,4a*R*,6*R*,8a*R*) is as shown in Scheme I. This amino acid has the same relative and absolute stereochemistry as the NMDA antagonists **7** and **8**.[11]

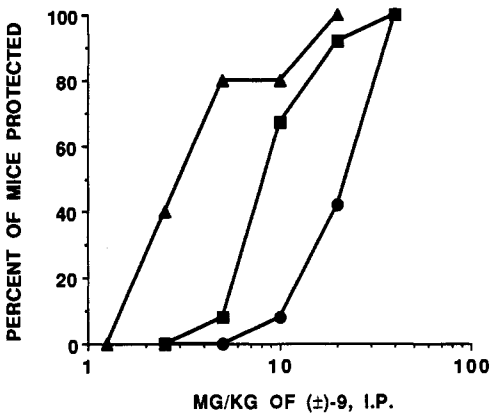


Figure 1. Figure 1. The effects of **9** in mice versus ATPA-induced rigidity (▲, $n = 5$), $ED_{50} = 3.6$ mg/kg (95% confidence interval (CI) = 1.7–6.3); versus maximal electroshock-induced convulsions (■, $n = 12$), $ED_{50} = 9.0$ mg/kg (95% CI = 6.8–11.8); and on the horizontal screen (●, $n = 12$), $ED_{50} = 19.6$ mg/kg (95% CI = 15.3–25.6). Compound **9** was administered by the intraperitoneal route in 30% polyethylene glycol 30 min prior to administration of 30 mg/kg (intravenous) of ATPA or maximal electroshock, or testing on the horizontal screen.

Conclusions. Recent data on **4** and **5** indicate that AMPA receptor antagonists should be useful as anticonvulsant and cerebroprotective agents, and this information has generated considerable interest in the development of this class of compounds. We have discovered a structurally novel, water-soluble (over a broad pH range), potent, and selective competitive AMPA antagonist **9** that is active in vivo following systemic administration. For example, intraperitoneal administration of **9** protects mice from convulsions induced by maximal electroshock and from rigidity induced by the bioavailable AMPA agonist **17**. These effects are observed at doses that are 2–5 times less than those required to produce impairment in the mouse on a horizontal screen assay. Therefore, **9** is a promising new candidate for development as a selective AMPA antagonist.

Acknowledgment. The authors would like to thank the Physical Chemistry Department of Lilly Research Laboratories for spectral data and elemental analyses.

Supplementary Material Available: Experimental detail and spectral analysis for the preparation of (±)-**9**, (−)-**9**, (+)-**9**, and **11**–**15** (4 pages). Ordering information is given on any current masthead page.

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