Articles

(3SR,4aRS,6SR,8aRS)-6-(1H-Tetrazol-5-yl)decahydroisoquinoline-3-carboxylic Acid, a Novel, Competitive, Systemically Active NMDA and AMPA Receptor Antagonist

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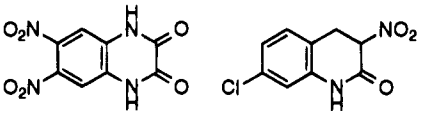
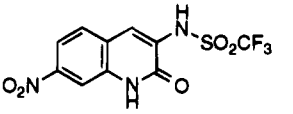
We report the synthesis and characterization of **6** (LY246492), which is a competitive *N*-methyl-D-aspartate (NMDA) and 2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propanoic acid (AMPA) receptor antagonist. Tetrazole-substituted amino acid **6** was prepared in four steps from the recently described aldehyde **7**. The optical isomers (−)-**6** and (+)-**6** were obtained from the same sequence of reactions using the corresponding isomers of **7**. The compound displaces both NMDA and AMPA receptor binding and antagonizes depolarizations in cortical slices evoked by both NMDA and AMPA. In mice and pigeons, the compound showed antagonism of responses mediated through NMDA and AMPA receptors. Using the resolved optical isomers of **6**, both NMDA and AMPA antagonist activities were found to reside in a single isomer, (−)-**6**.

Excitatory amino acid (EAA) antagonists are a promising line of therapy for the treatment of a number of central nervous system (CNS) disorders. Glutamic acid, which is the major excitatory neurotransmitter in the CNS, acts at certain subclasses of EAA receptors that are coupled to ion channels gating the influx of sodium and calcium ions. These "ionotropic" receptors are distinguished by the agonists that selectively activate them and include the *N*-methyl-D-aspartate (NMDA), 2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propanoic acid (AMPA), and kainic acid receptors.[1] Evidence now indicates that neuronal excitation mediated by glutamate acting at ionotropic receptors is involved in the pathophysiology of a number of acute and chronic neuronal disorders. Included among these disorders are cerebral ischemia,[2] spinal cord[3] and head trauma,[4] and Alzheimer's disease.[5] Thus, ionotropic EAA antagonists may prove useful in the treatment of these disorders. EAA antagonists are also anticonvulsants and may therefore be useful in the treatment of epilepsy.[6]

Most of the compounds that have been discovered as NMDA and AMPA antagonists are selective for a particular receptor. A limited number of examples are known where a single molecule is both an NMDA and an AMPA antagonist. They are typically quinoxalinediones, kynurenic acid derivatives, or related compounds, and their NMDA antagonist activity results from their activity at the glycine site on the NMDA receptor complex. For example, 6,7-dinitroquinoxaline-2,3-dione (**1**) has equal affinity for both the AMPA receptor (K_i = 0.36 μM) and the glycine site on the NMDA receptor (K_i = 0.39 μM).[7] Quinolinone **2** bound with high affinity to the glycine site on the NMDA receptor (IC_{50} = 0.41 μM versus [^{3}H]L-689,560 binding), was characterized as an NMDA and AMPA antagonist in a cortical slice preparation (K_bs = 6.7 and 9.2 μM, respectively), and showed anticonvulsant activity versus audiogenic seizures in DBA/2 mice, with an ED_{50} of 13.2 mg/kg.[8] This was the first example of a compound demonstrated to have both NMDA and AMPA antagonist activity that was also shown to be active in vivo. Quinolinone **3** bound with high affinity to the AMPA receptor (K_i = 0.6 μM versus [^{3}H]AMPA binding) and was shown to block depolarizations induced by either 30 μM AMPA (K_i = 0.37 μM) or 3 μM glycine/30 μM NMDA (K_i = 0.62 μM) in Xenopus oocytes injected with rat brain mRNA.[9]



1 **2**



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We have explored the medicinal chemistry of 6-substituted decahydroisoquinoline-3-carboxylates and have found both NMDA and AMPA receptor antagonists among this class of compounds. For example, the tetrazolylmethyl compound **4** (LY233536) is a potent NMDA antagonist.[10,11] Chain extension of this compound to the tetrazolylethyl compound **5** (LY215490) gave a potent AMPA receptor antagonist.[12] We discovered a compound (**6**, LY246492) during our structure–activity studies that possesses both NMDA and AMPA

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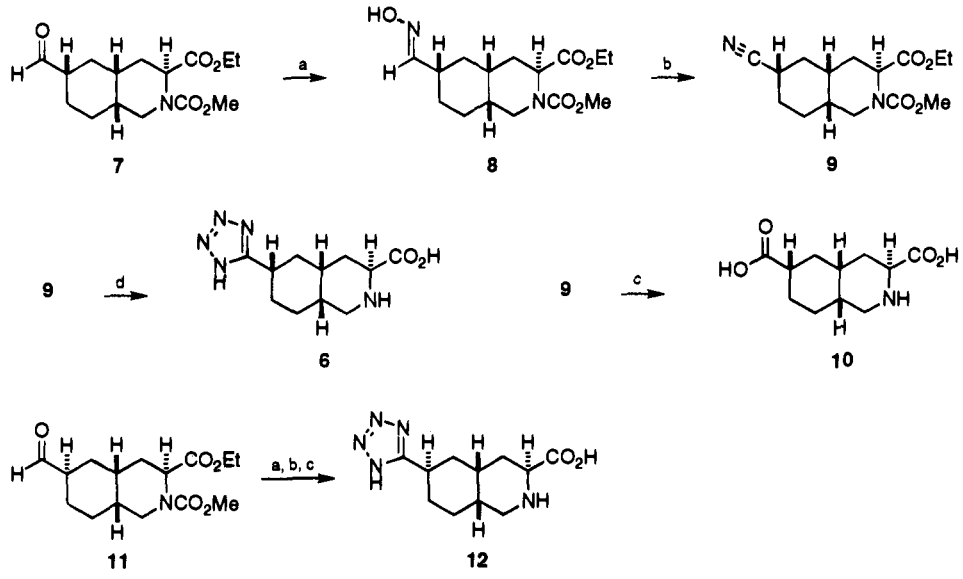
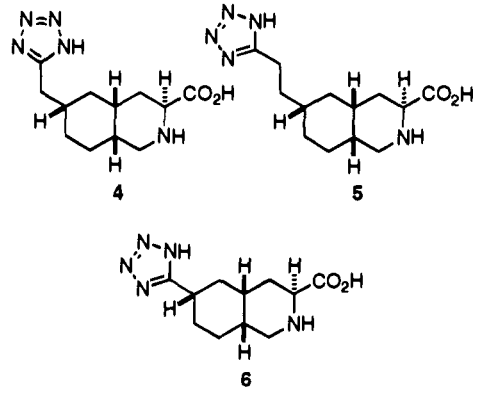
Scheme 1[a]



[a] (a) $NH_2OH \cdot HCl$, CH_2Cl_2, methanol, pyridine, room temperature; (b) $PhP(O)Cl_2$, CH_2Cl_2, pyridine, 0 °C to room temperature; (c) n-Bu_3SnN_3, 80 °C; 6 N HCl, reflux; Dowex 50-X8, 10% pyridine/water; (d) 5 N NaOH, methanol, reflux; 6 N HCl, reflux; Dowex 50-X8, 10% pyridine/water.

receptor antagonist activity within a single molecule. We describe herein the synthesis of the tetrazole substituted amino acid **6**, along with some structural analogues, and its pharmacological characterization as a competitive NMDA and AMPA antagonist.



Chemistry

Tetrazole-substituted amino acid **1** and the corresponding carboxylic acid analogue **11** were synthesized as shown in Scheme 1. Aldehyde **7**[13] was condensed at room temperature with hydroxylamine hydrochloride and pyridine in dichloromethane and methanol to afford an *E*/*Z* mixture of oximes **8**. This solution was concentrated, redissolved in dichloromethane and pyridine, and then treated with phenylphosphonic dichloride at 0 °C to afford the nitrile **9** in 86% yield. Treatment of the nitrile neat with 2 equiv of azido tri-*n*-butylstannane at 80 °C followed by hydrolysis with 6 N hydrochloric acid afforded amino acid **6**.[10] Alternatively, the nitrile **9** was directly hydrolyzed to afford the carboxy-substituted amino acid **10**. The same sequence of reactions on the C-6 epimeric aldehyde **11**[13] afforded the corresponding C-6 epimeric tetrazole-substituted amino acid **12**. The (−)-3*S*,4a*R*,6*S*,8a*R*- and (+)-3*R*,4a*S*,6*R*,-8a*S*-isomers of **6** were obtained from the same sequence of reactions using (−)-**7** or (+)-**7**, respectively.[13]

Results

Compounds were evaluated for their affinities at NMDA, AMPA, and kainic acid receptors by determining their ability to inhibit the binding of the selective radioligands [^{3}H]CGS 19755,[14] [^{3}H]AMPA,[15] and [^{3}H]-kainic acid,[16] respectively. Antagonist potency was determined in a cortical slice preparation,[17] where the compounds were evaluated for their ability to inhibit depolarizations induced by 40 μM NMDA, 40 μM AMPA, or 40 μM quisqualic acid (QUIS) and 10 μM kainic acid. Data for amino acids **6**, **10**, and **12** in these assays are shown in Table 1. Data on the NMDA antagonist **4**[10] and the AMPA antagonist **5**[12] are also provided in the table for comparison.

NMDA and AMPA antagonist activity were evaluated in vivo by determining the ability of these compounds to protect mice from lethality induced by a 200 mg/kg (ip) dose of NMDA,[18] to block maximal electroshock-induced convulsions in mice,[19] to block the characteristic rigidity induced in mice by a 30 mg/kg (iv) dose of the systemically active AMPA agonist 2-amino-3-(3-hydroxy-5-*tert*-butylisoxazol-4-yl)propanoic acid (ATPA),[20] to protect mice from clonic–tonic and tonic extensor convulsions incuded by a 12.5 nmol intracerebroventricular (icv) dose of NMDA or AMPA, and to reverse 10 mg/kg (im) NMDA-induced or 20 mg/kg (im) AMPA-induced suppression of responding in a schedule controlled behavioral paradigm in pigeons.[21] Compounds were administered intraperitoneally (ip) in mice and intramuscularly (im) in pigeons.

The tetrazole substituted amino acid **6** bound with moderate affinity to both NMDA and AMPA receptors, with IC_{50}s in selective radioligand binding assays of 1.6 ± 0.2 and 12.8 ± 0.3 μM, respectively. Weaker affinity was observed for binding to kainic acid receptors, with an IC_{50} of 31.8 ± 2.3 μM. In the cortical slice preparation, amino acid **6** antagonized depolarizations induced by NMDA and QUIS, with IC_{50}s of 7.5 ± 0.7 and 40.9 ± 5.2 μM, respectively. It did not, however, antagonize depolarizations due to kainic acid at doses up to 100

Table 1. Excitatory Amino Acid Receptor Binding Affinities and Antagonist Potencies of Decahydroisoquinoline Amino Acids

	IC_{50} (μM) versus Radioligand Binding at Ionotropic Excitatory Amino Acid Receptors[a,b]			IC_{50} (μM) versus Agonist-Induced Depolarizations in a Cortical Slice Preparation[c]		
compd	[^{3}H]CGS 19755	[^{3}H]AMPA	[^{3}H]Kainic Acid	NMDA	AMPA	Kainic Acid
(±)-**6**	1.6 ± 0.2	12.8 ± 0.3	31.8 ± 2.3	7.5 ± 0.7	40.9 ± 5.2[e]	>100
(−)-**6**	1.0[d]	5.7 ± 0.9	21.0[d]	4.1 ± 0.3	13.2 ± 2.4	>100
(+)-**6**	>100	>100	>100	>100	>100	>100
(±)-**10**	>100	>100	NT	>100	>100	>100
(±)-**12**	38.4 ± 9.1	>100	>100	65 ± 5.2	>100[e]	NT
(±)-**4**	0.82 ± 0.20[f]	84.5 ± 1.9[f]	>100[f]	1.4 ± 0.3[f]	>100[e,f]	>100[f]
(±)-**5**	26.4 ± 2.0[g]	4.8 ± 1.2[g]	247.0 ± 7.5[g]	61.3 ± 3[g]	6.0 ± 1.0[g]	31.7 ± 4.4[g]

[a] Affinity at NMDA receptors was determined using [^{3}H]CGS 19755; see ref 14. Affinity at AMPA receptors was determined using [^{3}H]AMPA; see ref 15. Affinity at kainic acid receptors was determined using [^{3}H]kainic acid; see ref 16. [b] All assays for affinity were run in triplicate, unless otherwise indicated. [c] See ref 16. [d] IC_{50} was the result of a single assay. [e] Tested versus quisqualic acid instead of AMPA. [f] Data from ref 10. [g] Data from ref 12.

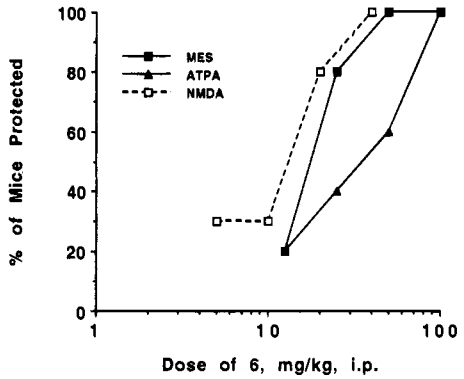


Figure 1. Protective effects of (±)-**6** in mice against maximal electroshock-induced seizures (MES), 30 mg/kg (iv) ATPA-induced lethality, or 200 mg/kg (ip) NMDA-induced lethality. Each data point is based on 10 mice/dose.

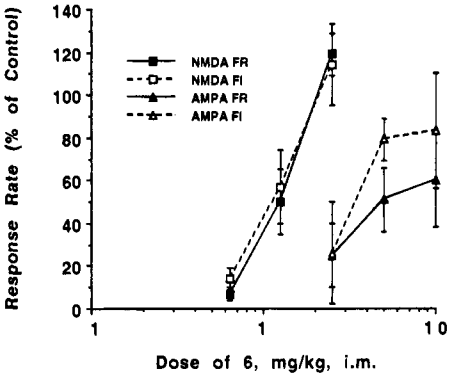


Figure 2. Antagonism by (±)-**6** of behavioral suppression produced by either 10 mg/kg of NMDA or 40 mg/kg of ATPA in pigeons responding under a Multiple FR, FI schedule of grain presentation. When administered alone, these doses of NMDA and ATPA produce virtually complete suppression (0–10% of control) of responding in both the FR and FI components of the multiple schedule. Each point is the mean and SEM of four birds.

μM. The amino acid **12**, which is epimeric at C-6 to **6**, was significantly less potent, showing only weak affinity at NMDA receptors (IC_{50} of 38.4 ± 9.1 μM) and weak antagonist activity versus NMDA in the cortical slice preparation (IC_{50} of 65 ± 5.2 μM). At concentrations up to 100 μM, **12** showed no affinity or activity at AMPA or kainic acid receptors, nor did it inhibit depolarizations due to QUIS or kainic acid. The carboxylic acid analogue **10** of amino acid **6** was inactive in both binding and cortical slice assays.

Affinity and antagonist activity at both NMDA and AMPA receptors reside in a single optical isomer of **6**. The (−)-3*S*,4a*R*,6*S*,8a*R*-isomer of **6** shows affinity at both NMDA and AMPA receptors, with IC_{50}s of 1.0 and 5.7 ± 0.9 μM, respectively, while the (+)-3*R*,4a*S*,6*R*,-8a*S*-isomer of **6** showed no significant inhibition of radioligand binding at concentrations up to 100 μM. Antagonist activity was found in the cortical slice preparation only for (−)-**6**, with IC_{50}s versus NMDA and AMPA of 4.1 ± 0.3 and 13.2 ± 2.4 μM, respectively.

In mice, amino acid (±)-**6** blocked NMDA-induced lethality and ATPA-induced rigidity with ED_{50}s of 19.4 and 44.5 mg/kg (ip), respectively (Figure 1). This compound was also effective in blocking MES-induced convulsions in mice, with an ED_{50} of 24.1 mg/kg (ip; Figure 1). Amino acid (±)-**6** blocked convulsions induced by NMDA and AMPA, with minimum effective doses of 50 mg/kg, ip against each agonist. Amino acid (±)-**6** dose-dependently reversed the rate suppressant effects of NMDA and AMPA in pigeons, with AD_{50}s of 1.3 and 4.8 mg/kg (im), respectively (Figure 2).

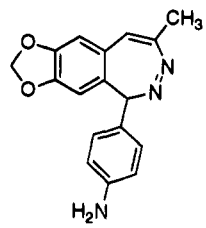
Discussion

In a series of decahydroisoquinoline-3-carboxylic acids substituted at C-6 with a tetrazole group that is separated from the rigid bicyclic nucleus by a carbon chain of varying lengths, we have discovered potent excitatory amino acid antagonists. In this series of amino acid antagonists, very subtle structural changes lead to distinct differences in activity. When the tetrazole group is separated from the nucleus by a one methylene spacer, as in **4**, the compound is a potent and selective NMDA antagonist. Add an additional methylene unit, as in the tetrazolylethyl compound **5**, and one obtains a potent and selective AMPA antagonist. However, if the tetrazole ring is connected directly to the hydroisoquinoline nucleus, the compound possesses both NMDA and AMPA antagonist activity. When amino acid **6** was resolved into its optical isomers, it was found that antagonist activity at both receptors resides within a single isomer. For **4**, **5**, and **6**, the relative and absolute stereochemistry of the four asymmetric centers is identical. Thus, there are many structural similarities in the amino acid recognition site of both the NMDA and AMPA receptors.

In our studies with these hydroisoquinoline amino acids, only **6** showed both NMDA and AMPA antagonist activity in vivo. While **5** has affinity at the NMDA receptor and shows NMDA antagonist activity in the cortical slice preparation (IC_{50}s of 26.4 ± 2.0 and 61.3 ± 3 μM, respectively), no NMDA antagonist activity is evident for this compound in vivo. No protection against

NMDA-induced lethality was observed for doses of **5** up to 320 mg/kg, which is about 90 times the ED_{50} of 3.6 mg/kg versus ATPA-induced rigidity in mice.[22]

Following systemic administration of **6** in mice, we observed evidence of antagonist activity at both NMDA and AMPA receptors. The NMDA-induced lethality assay in mice is highly specific for NMDA antagonists, regardless of the site at which they act on the NMDA receptor protein. Amino acid **6** inhibited this lethality at an ED_{50} of 19.4 mg/kg (ip), which is less potent than the selective NMDA antagonist **4** (MED = 2.5 mg/kg, ip). It was also effective in blocking the effects of the systemically active AMPA agonist ATPA. The substitution of the methyl group on the isoxazole ring of AMPA for the *tert*-butyl group in ATPA allows for better penetration of this amino acid into the CNS. In this paradigm, the characteristic muscular rigidity induced by intravenous administration of ATPA is blocked in a dose-dependent fashion by amino acid **6**, with an ED_{50} of 44.5 mg/kg (ip). For comparison, the noncompetitive AMPA antagonist **13** (GYKI 52466)[23] was equipotent in blocking this muscle rigidity, with an ED_{50} of 41.8 mg/kg, ip. For amino acid **6**, there is a 2-fold difference in mice between doses that antagonize NMDA receptor- and AMPA receptor-mediated responses. While not specific for either receptor, convulsions in mice induced by maximal electroshock are blocked by either NMDA or AMPA receptor antagonists. Amino acid **6** dose-dependently inhibited these convulsions, with an ED_{50} of 17.0 mg/kg (ip). This compound was also effective in blocking convulsions induced by icv injection of the EAA agonists NMDA and AMPA.



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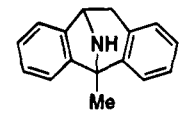
The combined NMDA and AMPA antagonist activity of amino acid **6** was also realized in a behavioral assay in pigeons. NMDA and AMPA suppress rates of responding in a dose-dependent manner in pigeons trained to peck for food in a schedule-controlled behavioral paradigm. When administered alone, almost complete suppression of response is observed with a 10 mg/kg dose of NMDA or a 20 mg/kg dose of AMPA. These rate suppressant effects of NMDA or AMPA are reversed by cotreatment with amino acid **6** at AD_{50}s of 1.3 and 4.8 mg/kg, im, respectively.

While many studies have demonstrated the therapeutic potential of selective NMDA and AMPA antagonists, little is known about the therapeutic potential of a combined NMDA/AMPA antagonist due to the limited availability and lack of in vivo potency of the existing agents. It is intruiging to ponder whether having both NMDA and AMPA antagonist activity in a single compound would be more beneficial (synergistic) or deleterious from a therapeutic point of view. Mouse culture cortical neurons subjected to oxygen and glucose deprevation showed enhanced neuroprotection when exposed to both an uncompetitive NMDA antagonist (**14**, MK-801) and a competitive AMPA antagonist (**15**, CNQX) compared to that with **14** alone (**15** alone was ineffective).[24] In a model of kindled seizures in rats, synergistic effects were observed in the coadministration of a competitive AMPA antagonist (NBQX, **16**) and a rapidly channel blocking NMDA receptor antagonist (memantine, **17**).[25] The combination of **16** and **17** was



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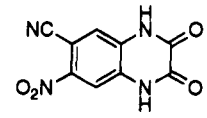

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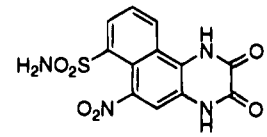

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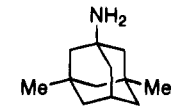

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more effective for increasing the after-discharge threshold than **16** alone, and **17** alone was inactive in this assay.[26] In a model of transient focal ischemia in spontaneously hypertensive rats (2 h of transient ischemia followed by 22 h of reperfusion) or transient severe forebrain ischemia in rats (four-vessel occlusion) where the AMPA antagonist **16** alone gave significant neuroprotection (as determined by measuring the volume of cortical infarction for the former and the extent of hippocampal CA1 damage in the latter), administration of **14** alone or a combination of **14** and **16** were ineffective.[26,27] In these models of in vivo neuroprotection, no synergistic effect of a combination of an NMDA and an AMPA antagonists was realized. In fact, the neuroprotective effects of the AMPA antagonist alone were lost during combined treatment. Studies with an agent such as **6** should more clearly elucidate the potential benefits or liabilities of a combination of both NMDA and AMPA antagonism.

The development of NMDA antagonists as therapeutic agents has been affected by the findings of Olney[28] and Fix,[29] who showed that competitive and noncompetitive PCP-like NMDA antagonists produce a characteristic vacuolization in neurons of the rat posterior cingulate/retrosplinial cortex. Upon short-term exposure, they demonstrated that this effect is reversible; however, prolonged exposure leads to cell death. This phenomenon was shown to correlate to increases in local cerebral glucose utilization, measured using 2-deoxyglucose autoradiography.[30] This effect has only been demonstrated in rats, and the physiological relevance of this phenomenon in humans remains unclear. These observations have generated questions regarding the safety of NMDA antagonists as neuroprotective agents in humans.[31]

It was recently demonstrated that competitive AMPA antagonists, such as **5** and **16**, do not produce increases in local cerebral blood glucose utilization; in fact, they decrease blood glucose utilization in many regions of the brain including the cingulate cortex.[32] It is therefore unlikely that a competitive AMPA antagonist would cause vacuolization and cell death in these neurons. An intriguing question that remains yet unanswered is what the effects of a compound such as **6**, which

possesses both NMDA and AMPA antagonist activity, would be on cingulate cortical neurons. One might speculate that the quiescence of neurons resulting from the AMPA antagonist activity inherent in a mixed antagonist such as **6** would counteract the neuronal excitation that results from its NMDA antagonist activity. Subsequent studies with a compound such as **6** will be required to answer this question.

We have prepared a novel amino acid that is both a competitive NMDA and a competitive AMPA receptor antagonist. Antagonist activity at both receptors is found within a single chemical entity, which may indicate that the recognition sites for acidic amino acids on NMDA and AMPA receptors share some structural similarities. This, however, is the first example of an acidic amino acid that has both NMDA and AMPA receptor antagonist activity that is also active in multiple assays in two animal species following systemic administration. Further investigation is necessary to elucidate the advantages that such a molecule might have in blocking neuronal degeneration mediated by activation of multiple EAA receptor subclasses.

Experimental Section

Ethyl (3*SR*,4a*RS*,6*SR*,8a*RS*)-6-Cyano-2-(methoxycarbonyl)decahydroisoquinoline-3-carboxylate (9). To a room temperature solution of 37.8 g (127 mmol) of **7**[13] in 570 mL of dichloromethane, 165 mL of methanol, and 20.5 mL of pyridine was added 8.8 g (127 mmol) of hydoxylamine hydrochloride. After 30 min at room temperature, the reaction mixture was concentrated in vacuo, an additional 300 mL of dichloromethane was added, and the mixture was again concentrated in vacuo to afford oxime **8**. The residue was dissolved in 870 mL of dichloromethane and 20.5 mL of pyridine, cooled to 0 °C, and treated dropwise with 36 mL (254 mmol) of phenylphosphonic dichloride. After stirring overnight at room temperature, the reaction mixture was quenched with 750 mL of saturated aqueous sodium bicarbonate, 1000 mL of ether was added, the phases were separated, and the organic phase was washed with 750 mL of 10% aqueous sodium bisulfate. The organic phase was dried ($MgSO_4$), filtered, and concentrated in vacuo. Preparative HPLC of the residue on a Waters Prep 2000 HPLC, loading in 25% ethyl acetate/toluene and eluting with a gradient of 5% ethyl acetate/hexane to 35% ethyl acetate/hexane, gave 31.6 g (86%) of **9**.

(3*SR*,4a*RS*,6*SR*,8a*RS*)-6-(1*H*-Tetrazol-5-yl)decahydroisoquinoline-3-carboxylic Acid ((±)-6). **9** (31.6 g, 107 mmol) and 79.2 g of azido tri-*n*-butylstannane in 30 mL of toluene were heated to 80 °C. After 7 days, the mixture was treated with 250 mL of 6 N hydrochloric acid and heated to 100 °C overnight. The mixture was cooled to room temperature and extracted six times with 200 mL each of ether, and the aqueous phase was then concentrated in vacuo. The residue was purified by ion-exchange chromatography on DOWEX 50-X8, eluting with 10% pyridine/water. The fractions containing the title compound (ninhydrin positive) were combined and concentrated in vacuo. The residue was diluted with 100 mL of water, concentrated in vacuo, diluted with water, and again concentrated in vacuo. The resulting solid was suspended in 150 mL of water, cooled to 0 °C for 4 h, then filtered, and washed with water, acetone, and ether. This material was dried in vacuo at 60 °C overnight to afford 16.4 g (61%) of (±)-**6**, mp 283 °C. Anal. ($C_{11}H_{17}N_5O_2 \cdot 0.5H_2O$) C, H, N.

(3*S*,4a*R*,6*S*,8a*R*)-6-(1*H*-Tetrazol-5-yl)decahydroisoquinoline-3-carboxylic acid ((−)-6) was prepared as above for racemic **6**. (−)-**6**: mp 244−247 °C; $[\alpha]_D = -22.8°$ ($c = 1$, 1 N HCl). Anal. ($C_{11}H_{17}N_5O_2 \cdot 1.25H_2O$) C, N; H: theory, 7.18; found, 6.75.

(3*R*,4a*S*,6*R*,8a*S*)-6-(1*H*-Tetrazol-5-yl)decahydroisoquinoline-3-carboxylic acid ((+)-6) was prepared as above for racemic **6**. (+)-**6**: mp 222−229 °C; $[\alpha]_D = +18.2°$ ($c = 1$, 1 N HCl). Anal. ($C_{11}H_{17}N_5O_2 \cdot 1.1H_2O \cdot 0.1C_3H_6O$) C, N; H: theory, 7.21; found, 6.58.

(3*SR*,4a*RS*,6*SR*,8a*RS*)-Decahydroisoquinoline-3,6-dicarboxylic Acid ((±)-10). A mixture of 3.8 g (12.9 mmol) of (±)-**9** in 30 mL of methanol and 50 mL of 5 N sodium hydroxide was heated to reflux overnight, then cooled, and concentrated in vacuo. The residue was dissolved in 30 mL of 6 N hydrochloric acid, heated to reflux for 3 h, then cooled, and concentrated in vacuo. The residue was purified by ion-exchange chromatography on DOWEX 50-X8, eluting with 10% pyridine/water. The fractions containing the title compound (ninhydrin positive) were combined and concentrated in vacuo. The resulting solid was suspended in water, filtered, washed with water, acetone, and ether, and then dried in vacuo at 60 °C overnight to afford 0.9 g (31%) of (±)-**10**, mp 279−280 °C. Anal. ($C_{11}H_{17}NO_4 \cdot 0.25\ H_2O$) C, H, N.

(3*SR*,4a*RS*,6*RS*,8a*RS*)-6-(1*H*-Tetrazol-5-yl)decahydroisoquinoline-3-carboxylic Acid ((±)-12). As for (±)-**6**, 0.8 g (5.4 mmol) of (±)-**11** and 0.37 g (5.4 mmol) of hydroxylamine hydrochloride in 25 mL of dichloromethane, 7 mL of methanol, and 0.9 mL of pyridine afforded an oxime that was dehydrated with 1.5 mL (2.1 g, 10.8 mmol) of phenylphosphonic dichloride in 20 mL of dichloromethane and 0.9 mL of pyridine to afford 0.9 g (58%) of (3*SR*,4a*RS*,6*RS*,8a*RS*)-6-cyano-2-(methoxycarbonyl)decahydroisoquinoline-3-carboxylate. A mixture of 0.8 g (2.8 mmol) of this nitrile and 1.8 g (5.5 mmol) of azidotri-*n*-butylstannane neat was heated at 80 °C for 3 days and as for (±)-**6** afforded 0.25 g (36%) of (±)-**12**, mp 241−242 °C. Anal. ($C_{11}H_{17}N_5O_2 \cdot 2.0H_2O$) C, H, N.

ATPA-Induced Rigidity in Mice. A 30 mg/kg intravenous dose of ATPA produced muscle rigidity in 100% of the mice (20−25 g; Charles River Laboratories, Portage, MI) that were administered vehicle alone (30% PEG). Experimental compounds were dissolved in 30% PEG and administered intraperitoneally 30 min prior to challenge with ATPA. After treatment with various doses of experimental compound, mice that did not exhibit ATPA-induced rigidity were scored as protected. The percent of mice protected at each dose of experimental compound was determined using 10 mice per dose.

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