5-(*N*-Oxyaza)-7-substituted-1,4-dihydroquinoxaline-2,3-diones: Novel, Systemically Active and Broad Spectrum Antagonists for NMDA/glycine, AMPA, and Kainate Receptors

Sui Xiong Cai,*,† Jin-Cheng Huang,† Stephen A. Espitia,† Minhtam Tran,† Victor I. Ilyin,† Jon E. Hawkinson,† Richard M. Woodward,† Eckard Weber,† and John F. W. Keana†,‡

CoCensys Inc., 201 Technology Drive, Irvine, California 92618, and Department of Chemistry, University of Oregon, Eugene, Oregon 97403

Received June 16, 1997[⊗]

A group of 5-aza-7-substituted-1,4-dihydroquinoxaline-2,3-diones (QXs) and the corresponding 5-(*N*-oxyaza)-7-substituted QXs were prepared and evaluated as antagonists of ionotropic glutamate receptors. The *in vitro* potency of these QXs was determined by inhibition of [^{3}H]-5,7-dichlorokynurenic acid ([^{3}H]DCKA) binding to *N*-methyl-D-aspartate (NMDA)/glycine receptors, [^{3}H]-(*S*)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid ([^{3}H]AMPA) binding to AMPA receptors, and [^{3}H]kainate ([^{3}H]KA) binding to KA receptors in rat brain membranes. 5-(*N*-Oxyaza)-QXs **12a**−**e** all have low micromolar or submicromolar potency for NMDA/glycine receptors and low micromolar potencies for AMPA and KA receptors. QXs **12a**−**e** display 2−12-fold selectivity for NMDA/glycine receptors compared to AMPA receptors, and ∼2-fold difference between AMPA and KA potency. In contrast to other QXs that either show high selectivity for NMDA (such as ACEA 1021) or AMPA (such as NBQX) receptors, these molecules are broad spectrum antagonists of ionotropic glutamate receptors. 7-Nitro-5-(*N*-oxyaza)-QX (**12e**) is the most potent inhibitor among **12a**−**e**, having IC_{50} values of 0.69, 1.3, and 2.4 μM at NMDA, AMPA, and KA receptors, respectively. In functional assays on glutamate receptors expressed in oocytes by rat cerebral cortex poly(A^+) RNA, 7-chloro-5-(*N*-oxyaza)-QX (**12a**) and 7-nitro-5-(*N*-oxyaza)-QX (**12e**) have K_b values of 0.63 and 0.31 μM for NMDA/glycine receptors, and are 6- and 4-fold selective for NMDA over AMPA receptors, respectively. 5-(*N*-Oxyaza)-7-substituted-QXs **12a**−**e** all have surprisingly high *in vivo* potency as anticonvulsants in a mouse maximal electroshock-induced seizure (MES) model. 7-Chloro-5-(*N*-oxyaza)-QX (**12a**), 7-bromo-5-(*N*-oxyaza)-QX (**12b**), and 7-methyl-5-(*N*-oxyaza)-QX (**12c**) have ED_{50} values of 0.82, 0.87, and 0.97 mg/kg iv, respectively. The high *in vivo* potency of QXs **12a**−**e** is particularly surprising given their low log *P* values (∼ −2.7). Separate studies indicate that QXs **12a** and **12e** are also active *in vivo* as neuroprotectants and also have antinociceptive activity in animal pain models. In terms of *in vivo* activity, these 5-(*N*-oxyaza)-7-substituted-QXs are among the most potent broad spectrum ionotropic glutamate antagonists reported.

Introduction

Glutamate is the major excitatory neurotransmitter in the mammalian central nervous system (CNS),[1] and its interactions with membrane receptors play a critical role in nearly every aspect of brain function, including cognition, memory, and sensation.[2] Glutamate receptors are classified into two main categories: ionotropic receptors, which are ligand-gated ion channels,[3] and metabotropic receptors, which are coupled to intracellular signal transduction pathway via G-proteins.[4] Ionotropic glutamate receptors are grouped into three classes based on their sensitivity to selective agonists: *N*-methyl-D-aspartate (NMDA) receptors, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, and kainate (KA) receptors.[5]

Many neurologic disorders, including acute conditions such as stroke and epilepsy, as well as chronic conditions such as Huntington's disease and amyotrophic lateral sclerosis, are believed to be due, at least in part, to overactivation of ionotropic glutamate receptors. One consequence of this overactivation is to cause excessive influx of Ca^{2+} ions into neurons, which subsequently triggers degeneration and death of the cells in a process known as excitotoxicity.[6,7] Antagonists of NMDA and AMPA receptors, in particular, are expected to be useful for the treatment of neurological conditions involving an excitotoxic component. Indeed, many NMDA and AMPA antagonists have been demonstrated to protect the CNS from excitotoxicity in animal models, and several are currently undergoing clinical evaluation to assess their safety and efficacy.[8]

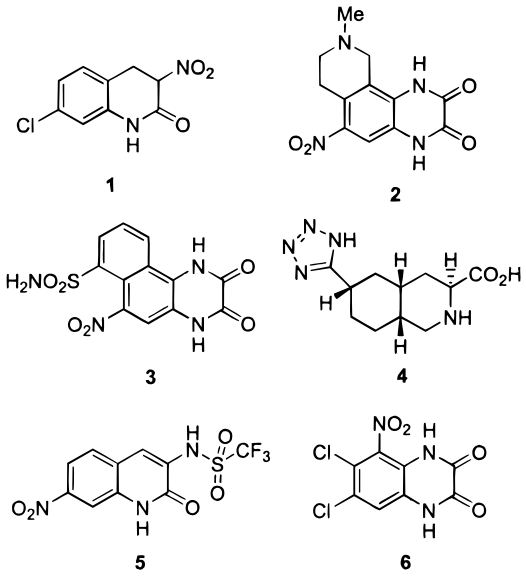
The development of ligands that interact with NMDA receptors has been a focus of research in recent years and has resulted in the discovery of many types of antagonists. These compounds can be divided into four broad classes based on the sites of interaction with the NMDA receptor channel complex:[9] (a) competitive antagonists for the glutamate binding site, (b) noncompetitive antagonists for the ion channel (channel blockers), (c) antagonists for the glycine coagonist site, and (d) allosteric antagonists for the ifenprodil modulatory site. In comparison, there are relatively few types of AMPA receptor antagonists. These compounds can be classified into two groups:[10] (a) competitive antagonist for the glutamate site[11−13] and (b) noncompetitive allosteric antagonists.[14,15]

Recently there were reports suggesting that combination treatment, involving antagonism of both NMDA

† CoCensys.
‡ University of Oregon.
[⊗] Abstract published in *Advance ACS Abstracts,* September 15, 1997.

S0022-2623(97)00396-8 CCC: $14.00

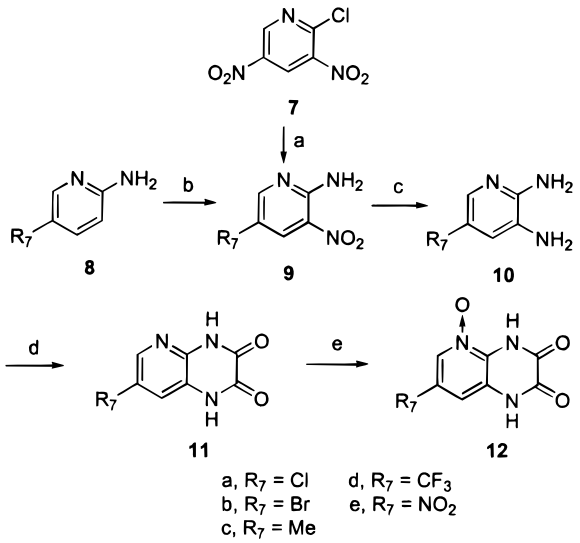
Chart 1



and AMPA receptors, might increase efficacy of drugs as anticonvulsants and analgesics over treatment with NMDA antagonists or AMPA antagonists alone.[10,16] For example, it was shown that the AMPA receptor antagonists GYKI 52466 and NBQX separately enhanced the protective effects of the NMDA receptor antagonists MK-801 and D-CPP-ene against electroconvulsions, and *vice versa*, MK-801 or D-CPP-ene reduced the ED_{50} values of both GYKI 52466 and NBQX in the maximal electroshock (MES) assay.[17] In addition, studies suggest that NMDA, AMPA, and KA receptors are all involved in peripheral pain transmission and that antagonism of multiple types of glutamate receptor might be necessary to achieve effective pain relief.[18,37] While NMDA receptor antagonists such as MK-801 are active as neuroprotectants in animal models of focal ischemia, AMPA antagonists such as NBQX are effective in both focal and global ischemia models.[19] Compounds with combined NMDA and AMPA receptor antagonist activities might therefore be therapeutically useful as anticonvulsants, analgesics, and neuroprotectants.

Several systemically active compounds with combined NMDA and AMPA receptor antagonist activity have been reported. 7-Chloro-3-nitro-3,4-dihydro-2(1*H*)-quinolone (**1**) is an NMDA/glycine ($K_b = 6.7\ \mu M$) and AMPA ($K_b = 9.2\ \mu M$) receptor antagonist which has anticonvulsant activity in DBA/2 mice ($ED_{50} = 13.2$ mg/kg).[20] 1,4,7,8,9,10-Hexahydro-9-methyl-6-nitropyrido[3,4-*f*]quinoxaline-2,3-dione (PNQX, **2**) has a broad binding profile at the NMDA/glycine ($IC_{50} = 0.37\ \mu M$), AMPA ($IC_{50} = 0.063\ \mu M$), and KA ($IC_{50} = 0.36\ \mu M$) receptors. PNQX was active against MES with an ED_{50} value of 0.44 mg/kg and was significantly more potent than NBQX (**3**) ($ED_{50} = 13.1$ mg/kg). NBQX has a similar potency as PNQX at AMPA receptors but is inactive at NMDA/glycine receptors.[21] This suggests that broad spectrum glutamate antagonists might be superior to selective AMPA/KA antagonists. Ornstein *et al.* reported recently that LY246492 (**4**) is a systemically active competitive NMDA ($IC_{50} = 1.6\ \mu M$) and AMPA ($IC_{50} = 12.8\ \mu M$) receptor antagonist. LY246492 is effective in blocking MES-induced convulsions in mice with an ED_{50} value of 24.1 mg/kg.[22] In addition, a class of quinolones represented by **5** (Chart 1) is reported to have balanced

Scheme 1[a]



[a] (a) NH_4OH; (b) HNO_3/H_2SO_4; (c) $(NH_4)_2S$, or $SnCl_2$/EtOH, or H_2/Pd, or H_2/Raney Ni; (d) $(HO_2C)_2$/2 N HCl or $(EtO_2C)_2$; (e) MCPBA or CF_3CO_3H.

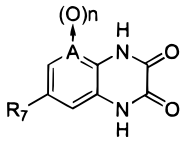
NMDA/glycine ($IC_{50} = 0.62\ \mu M$) and AMPA ($IC_{50} = 0.63\ \mu M$) antagonist activities.[23] The *in vivo* activity of these quinolones, however, was reported to be disappointing due to poor brain bioavailability.

We have recently reported on the synthesis and SAR of 1,4-dihydroquinoxaline-2,3-diones (QXs) leading to the discovery of 6,7-dichloro-5-nitro-QX (ACEA 1021, **6**).[24] QX **6** is a selective, highly potent and systemically active NMDA/glycine antagonist.[25] To further explore the SAR of QXs, we sought to replace the carbon atom in the 5-position of QX by a nitrogen atom and its corresponding *N*-oxide.[26] These changes were designed to reduce log *P* and improve aqueous solubility. Herein we report that 5-(*N*-oxyaza)-7-substituted-QXs, as represented by 7-chloro-5-(*N*-oxyaza)-QX (**12a**) and 7-nitro-5-(*N*-oxyaza)-QX (**12e**), are systemically active, broad spectrum antagonists of NMDA/glycine, AMPA, and KA receptors.

Chemistry

The 5-aza-7-substituted-QXs were prepared in general from the appropriate 2-amino-5-substituted-pyridines (Scheme 1). Direct nitration of 2-amino-5-chloropyridine (**8a**) gave 2-amino-5-chloro-3-nitropyridine (**9a**),[27] which was reduced to 2,3-diamino-5-chloropyridine (**10a**) via hydrogenation using Raney Ni as catalyst. The diamine **10a** was cyclized with oxalic acid to give 5-aza-7-chloro-QX (**11a**). 2-Amino-5-bromo-3-nitropyridine (**9b**) was best reduced by $SnCl_2$ to give 2,3-diamino-5-bromopyridine without concomitant reduction of the bromo group. Aza-QX **11c,d** was similarly prepared from 2-amino-5-methylpyridine (**8c**) and 2-amino-5-trifluoromethylpyridine (**8d**), respectively. 2,3-Diamino-5-nitropyridine (**10e**) was obtained from nucleophilic substitution of 2-chloro-3,5-dinitropyridine (**7**) by ammonium hydroxide to give 2-amino-3,5-dinitropyridine (**9e**) followed by selective reduction of the 3-nitro group using $(NH_4)_2S$. Oxidation of aza-QXs **11a**−**c** using *m*-chloroperbenzoic acid (MCPBA) gave the corresponding (*N*-oxyaza)-QXs **12a**−**c**. Aza-QXs **11d,e**, owing to the presence of the strong electron-withdrawing trifluoromethyl and nitro group in the

Table 1. SAR of 5-Aza- and 5-(*N*-Oxyaza)-QXs at NMDA, AMPA, and KA Receptors



				IC$_{50}$,[a] μM		
no.	A	*n*	R_7	[^{3}H]DCKA	[^{3}H]AMPA	[^{3}H]Kainate
11a	N	0	Cl	2.2 ± 0.4	38 ± 2	22 ± 3
11b	N	0	Br	4.1 ± 0.5	32 ± 3	16 ± 2
11c	N	0	Me	41 ± 2	67 ± 12	49 ± 10
11d	N	0	CF_3	1.9 ± 0.3	18 ± 2	16 ± 1
11e	N	0	NO_2	1.1 ± 0.1	3.3 ± 0.8	4.4 ± 0.3
12a	N	1	Cl	0.82 ± 0.11	9.9 ± 0.5	5.3 ± 0.4
12b	N	1	Br	1.0 ± 0.2	6.7 ± 0.7	3.4 ± 0.8
12c	N	1	Me	5.9 ± 1.1	14 ± 1	11.9 ± 0.4
12d	N	1	CF_3	1.05 ± 0.03	5.9 ± 1.1	7.5 ± 1.4
12e	N	1	NO_2	0.69 ± 0.06	1.3 ± 0.8	2.4 ± 0.3
13a	CH	0	Cl	1.8 ± 0.3	52 ± 14	52 ± 5
13e	CH	0	NO_2	3.8 ± 0.5	7.7 ± 0.3	15 ± 4

[a] Affinity is expressed as IC$_{50}$ estimates for inhibition of [^{3}H]DCKA, [^{3}H]AMPA, and [^{3}H]KA binding to rat brain membranes, respectively. Values are means ± SEMs of at least three independent experiments.

7-position, were best oxidized with CF_3CO_3H (prepared *in situ* from $(CF_3CO)_2O$ and H_2O_2) to give the corresponding (*N*-oxyaza)-QXs **12d,e**.

Pharmacology

The potency of aza-QXs and (*N*-oxyaza)-QXs for NMDA/glycine receptor was measured by displacement of [^{3}H]DCKA binding to rat brain cortical membranes as described previously.[28] The potency of the QXs at the AMPA receptor was measured by displacement of [^{3}H]AMPA,[29] and the potency at the KA receptors was measured by displacement of [^{3}H]KA.[30] Potency is expressed as the IC$_{50}$ values for inhibition of radioligand binding to rat brain membranes. Values are given as the mean ± SEM of at least three independent experiments. For selected QXs, potencies for NMDA/glycine receptors and for AMPA receptors were determined electrophysiologically in *Xenopus* oocytes expressing rat brain poly(A)$^+$ RNA or the cloned rat NMDA receptor (NR)1A/2C subunit combination.[31] Apparent antagonist dissociation constants (K_b values) were estimated by assuming competitive inhibition and assaying suppression of membrane current responses elicited by fixed concentrations of agonist: 1 μM glycine plus 100 μM glutamate for NMDA receptors and 10 μM AMPA for AMPA receptors.[28] Anticonvulsant activity of QXs was measured in a mouse MES model by iv administration.[32−34] Activity in the MES model was used as an indirect estimate of systemic bioavailability.

Results and Discussion

The potencies of the 5-aza- and corresponding 5-(*N*-oxyaza)-QXs as antagonists at NMDA/glycine, AMPA, and KA receptors are given in Table 1. Aza-QXs **11a,b** and **11d,e** all have low micromolar potency at the NMDA/glycine receptors. 5-Aza-7-nitro-QX (**11e**) is the most potent inhibitor with an IC$_{50}$ value of 1.1 μM. 5-Aza-7-methyl-QX (**11c**) is the least potent inhibitor and is about 10 times less potent than any of other aza-QXs. This implies that potency is dependent on an electron-withdrawing group in the 7-position for the 5-aza-7-substituted-QXs. 5-Aza-7-chloro-QX (**11a**) and the corresponding QX **13a**[24] have similar potencies, while aza-QX **11e** is about 3-fold more potent than the corresponding QX **13e**.[24] This indicates that the introduction of a nitrogen in the 5-position dose not have a large effect on NMDA/glycine potency.

Aza-QXs **11a,b** and **11d** are about 10-fold less potent at AMPA than at NMDA/glycine receptors. 5-Aza-7-nitro-QX (**11e**) is the only compound with low micromolar potency at AMPA receptors (3.3 μM) and is just 3-fold less potent than at NMDA/glycine receptors. The nitro group in the 7-position is therefore preferred for AMPA receptor activity, as has been found in other series of AMPA antagonists.[21] In parallel with their potencies for NMDA/glycine receptors, aza-QX **11a** and QX **13a**, as well as aza-QX **11e** and QX **13e**, have comparable potencies at AMPA receptors, indicating that the introduction of a nitrogen in the 5-position has little effect on the potency.

Interestingly, the KA receptor potencies of aza-QXs **11a**−**e** are all within 2-fold of their potency at AMPA receptors. Aza-QX **11e** is again the most potent inhibitor with IC$_{50}$ value of 4.4 μM. The nitro group in the 7-position is therefore preferred for KA receptor activity. Aza-QX **11a** is about 2-fold more potent than QX **13a**, and aza-QX **11e** is about 3-fold more potent than QX **13e**. This indicates that a nitrogen in the 5-position has little effect on the potency of QXs at KA receptors.

(*N*-Oxyaza)-QXs **12a**−**e** all have low micromolar or submicromolar potency at NMDA/glycine receptors. 7-Nitro-5-(*N*-oxyaza)-QX (**12e**) is the most potent inhibitor with an IC$_{50}$ value of 0.69 μM. 7-Methyl-5-(*N*-oxyaza)-QX (**12c**) is the least potent inhibitor, implying that an electron-withdrawing group in the 7-position is also important for the 5-(*N*-oxyaza)-QXs. (*N*-Oxyaza)-QXs **12a**−**e** are 2−7-fold more potent than the corresponding aza-QXs **11a**−**e**, indicating that the polar and electron-withdrawing *N*-oxide group in the 5-position is favorable for the potency of QXs at NMDA/glycine receptors.

(*N*-Oxyaza)-QXs **12a**−**e** all have low micromolar potencies at AMPA receptors. 7-Nitro-5-(*N*-oxyaza)-QX (**12e**) is the most potent AMPA receptor antagonist (IC$_{50}$ 1.3 μM), again confirming that a nitro group in the 7-position is preferred for AMPA receptor activity. (*N*-Oxyaza)-QXs **12a**−**e** are 3−5-fold more potent than the corresponding aza-QXs **11a**−**e** at the AMPA receptor, indicating that the *N*-oxide group in the 5-position is also favorable for the potency of QXs at AMPA receptors. (*N*-Oxyaza)-QXs **12a**−**e** are about 2−12-fold less potent at AMPA receptors than at NMDA/glycine receptors. (*N*-Oxyaza)-QX **12e** shows the best balance between NMDA/glycine and AMPA receptor potencies (2-fold). In comparison, the broad spectrum antagonist LY246493 (**4**) is 8-fold more active at NMDA/glycine receptors than at AMPA receptors,[22] and PNQX is 6-fold selective for AMPA vs NMDA/glycine receptors.[21]

(*N*-Oxyaza)-QXs **12a**−**e** all have low micromolar potencies at KA receptors. As is the case with NMDA/glycine and AMPA receptors, 7-nitro-5-(*N*-oxyaza)-QX (**12e**) is the most potent antagonist (IC$_{50}$ = 2.4 μM). (*N*-Oxyaza)-QXs **12a**−**e** are 2−5-fold more potent than the corresponding aza-QXs **11a**−**e** at KA receptors, indicating that the 5-*N*-oxide group is also favorable for

Table 2. Functional Antagonism of 5-Aza- and 5-(*N*-Oxyaza)-QXs at NMDA/glycine and AMPA Receptors Expressed in *Xenopus* Oocytes

	K_b (μM)		selectivity	
no.	NMDA/glycine	AMPA[b]	for NMDA[c]	n[d]
11e	0.43[f] (0.39−0.46)	1.4 (1.2−1.5)	3.3	3, 3
12a	0.63[a] (0.56−0.71)[e]	3.9 (3.4−4.4)	6.2	4, 5
12e	0.31[f] (0.29−0.33)	1.3 (1.2−1.4)	4.2	3, 5

[a] Inhibition of NMDA/glycine receptors was measured in oocytes expressing rat cerebral cortex poly(A)$^+$ RNA. K_b values at glycine binding sites were estimated, assuming simple competitive antagonism, from inhibition of currents elicited by 1 μM glycine and 100 μM glutamate. [b] Inhibition of AMPA receptors was measured in oocytes expressing rat cerebral cortex poly(A)$^+$ RNA. K_b values at glutamate binding site were estimated from inhibition of currents elicited by 10 μM AMPA. [c] The steady-state selectivity index for inhibition of NMDA/glycine receptors was estimated by dividing K_b(AMPA) by K_b(NMDA/glycine). [d] Indicates the number of independent experiments (cells examined); numbers refer to NMDA/glycine and AMPA, respectively. [e] Numbers in parentheses are 95% confidence intervals adjusted to the linear scale. [f] Inhibition of NMDA/glycine receptors was measured in oocytes expressing the cloned rat NMDA receptor subunit combination NR1A/2C.

potency. Similar to the case with aza-QXs **11a**−**e**, (*N*-oxyaza)-QXs **12a**−**e** display less than 2-fold difference in potencies at KA and AMPA receptors.

Functional antagonism of AMPA receptors by aza-QX **11e** and (*N*-oxyaza)-QXs **12a** and **12e** and antagonism of NMDA/glycine receptor by **12a** was determined electrophysiologically in *Xenopus* oocytes expressing rat cerebral cortex poly(A)$^+$ RNA. Functional antagonism of NMDA/glycine receptors by **11e** and **12e** was determined in oocytes expressing the cloned rat NMDA receptor subunit combination NR1A/2C. The potencies of inhibition (K_b values) obtained from the functional assay (Table 2) are either very close to (for **12a**, NMDA/glycine, and **12e**, AMPA) or about 2-fold more potent (for **11e**, NMDA/glycine and AMPA; **12a**, AMPA; and **12e**, NMDA/glycine) than the potencies measured in the binding assay. On the basis of the functional assay, there is a 6-fold difference between NMDA/glycine potency (0.63 μM) and AMPA receptor (3.9 μM) for **12a** and a 4-fold difference between NMDA/glycine potency (0.31 μM) and AMPA potency (1.3 μM) for **12e**. 7-Nitro-5-(*N*-oxyaza)-QX (**12e**) is about 2-fold more potent than 7-chloro-5-(*N*-oxyaza)-QX (**12a**) at the NMDA/glycine receptor and 3-fold more potent than **12a** at the AMPA receptor. Interestingly, aza-QX **11e** and the corresponding (*N*-oxyaza)-QX **12e** have similar potency at the NMDA/glycine and AMPA receptors in the functional assay.

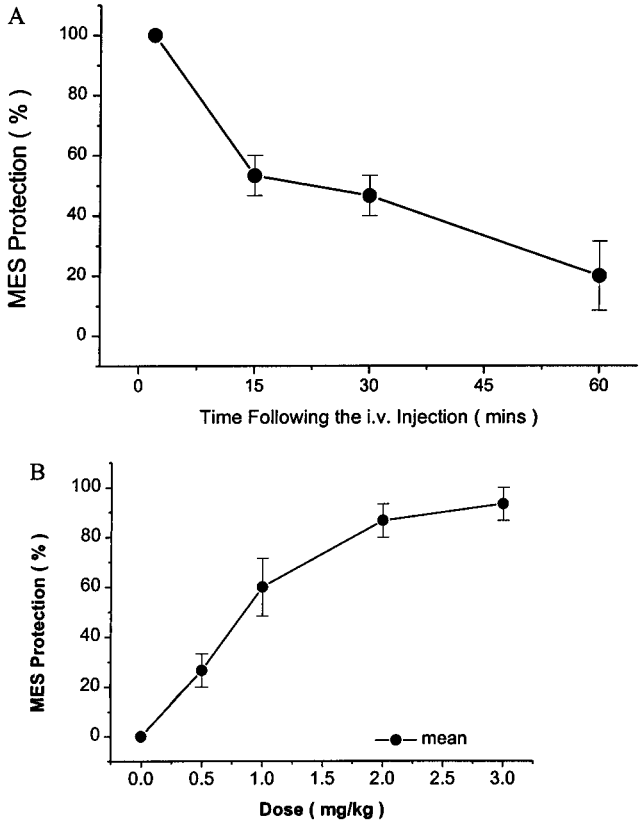
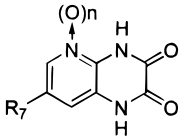


Figure 1. (A) Time course for (*N*-oxyaza)-QX **12a** in the maximal electroshock model (MES). QX **12a** was administrated intravenously (iv) in a dose of 5 mg/kg (three groups of eight animals per time point). (B) Dose−response curve for QX **12a** in the MES model. QX **12a** was administrated iv 2 min before delivery of the electroshock (three groups of eight animals per dose).

(*N*-Oxyaza)-QXs **12a**−**e** were tested in the maximal electroshock-induced seizures (MES) model to assess systemic bioavailability and were all found to have surprisingly high activity as anticonvulsants (Table 3). Figure 1A shows the time course for 7-chloro-5-(*N*-oxyaza)-QX (**12a**) in the MES model and serves as a prototype for the other compounds. The peak effect was at ∼2 min after iv administration. At 15 min, 50% of protection was lost, and at 60 min, there were only low levels of protection. Dose−response curves for all QXs

Table 3. Anticonvulsant Activity (MES) of QXs in Mouse



			IC_{50} μM		MES ED_{50},	
no.	n	R_7	[^{3}H]DCKA	[^{3}H]AMPA	mg/kg	log P
11e	0	NO_2	1.1 ± 0.1	3.3 ± 0.8	4.5 (3.8−7.9)	−0.97[a]
12a	1	Cl	0.82 ± 0.11	9.9 ± 0.5	0.82 (0.54−1.3)	−2.66[a]
12b	1	Br	1.0 ± 0.2	6.7 ± 0.7	0.87 (0.60−1.3)	−2.48[b]
12c	1	Me	5.9 ± 1.1	14 ± 1	0.97 (0.64−1.3)	−2.91[b]
12d	1	CF_3	1.05 ± 0.03	5.9 ± 1.1	6.3 (4.3−9.0)	−2.35[b]
12e	1	NO_2	0.69 ± 0.06	1.3 ± 0.8	4.6 (3.1−7.0)	−3.31[b]
6			0.0059 ± 0.001[c]	1.5 (1.4−1.6)[d]	4.0 (3.2−5.0)[c]	0.21[a]

[a] Measured by the shake-flask method in octanol vs pH 7.4 buffer. [b] Calculated on the basis of the log P value of QX **12a**. [c] Data from ref 34. [d] Data (K_b) from ref 24.

were measured at 2 min (Figure 1B) after administration of drugs. 7-Chloro-5-(*N*-oxyaza)-QX (**12a**), 7-bromo-5-(*N*-oxyaza)-QX (**12b**), and 7-methyl-5-(*N*-oxyaza)-QX (**12c**) were all highly potent anticonvulsants with ED_{50} values of 0.82, 0.87, and 0.97 mg/kg, respectively. 5-(*N*-Oxyaza)-7-(trifluoromethyl)-QX (**12d**) and 7-nitro-5-(*N*-oxyaza)-QX (**12e**) were also active *in vivo* with ED_{50} values of 6.2 and 4.6 mg/kg, respectively. By way of comparison, ACEA 1021 (QX **6**), which has a K_b of ~6 nM at NMDA/glycine receptors and 1.5 μM at AMPA receptors, has an ED_{50} of 4 mg/kg as an anticonvulsant in the same model.[34] These results imply that (*N*-oxyaza)-QXs **12a**−**e** have better central bioavailability than that of ACEA 1021 and other structurally related NMDA and AMPA antagonists.[21,32−34] The more balanced antagonism of (*N*-oxyaza)-QX **12a**−**e** at NMDA and AMPA receptors compared to QX **6** may also contribute to the high *in vivo* activity. This is consistent with the observation that the broad spectrum glutamate antagonist PNQX (ED_{50} = 0.44 mg/kg) is a better anticonvulsant than the selective AMPA antagonist NBQX (ED_{50} = 13.1 mg/kg).[21] We also note that (*N*-oxyaza)-QX (**12a**) is much less bound to plasma proteins (70−75% bound) than is QX **6** (~99% bound),[35] which almost certainly contributes to the high *in vivo* potency of **12a** and other (*N*-oxyaza)-QXs.

It is interesting to note that the potent anticonvulsant effects of these (*N*-oxyaza)-QXs runs counter to log *P* considerations. 7-Chloro-5-(*N*-oxyaza)-QX (**12a**) has a log *P* value of −2.66 as measured by the shake-flask method in octanol vs pH 7.4 buffer. On the basis of the log *P* of **12a**, 7-bromo-5-(*N*-oxyaza)-QX (**12b**), 7-methyl-5-(*N*-oxyaza)-QX (**12c**), 7-nitro-5-(*N*-oxyaza)-QX (**12e**) and 5-(*N*-oxyaza)-7-(trifluoromethyl)-QX (**12d**) have calculated log *P* values of −2.48, −2.91, −3.31, and −2.35, respectively. The two (*N*-oxyaza)-QXs (**12b** and **12c**) which have log *P* values closer to that of **12a** have similar anticonvulsant activities as that of **12a**, whereas compounds **12d** and **12e**, which have log *P* values slightly higher (**12d**) or lower (**12e**) than that of **12a**, are 7 and 5 times less active than **12a**. These data seem to suggest that, for this group of 5-(*N*-oxyaza)-7-substituted-QXs, a log *P* value around −2.7 might be optimal for *in vivo* activity. In general, a log *P* value of ~2 is considered to be optimal for CNS drugs.[36]

As expected from the low log *P* value, the (*N*-oxyaza)-QXs consistently have better aqueous solubility than the corresponding QXs and aza-QXs. Though no systematic/quantitative measurements were made, (*N*-oxyaza)-QXs **12a**−**e** are all readily soluble in 0.05 M tris(hydroxymethyl)aminomethane (Tris) aqueous solution. For example, (*N*-oxyaza)-QXs **12a** can be dissolved in 0.05 M aqueous Tris at concentrations up to 20 mg/mL. In contrast, most of the corresponding aza-QXs, except 5-aza-7-nitro-QX (**11e**), could not be dissolved in 0.05 M Tris at concentrations >1 mg/mL. Indeed, most of the aza-QXs were not tested in the MES assay because they could not be formulated into a Tris aqueous solution for iv injection.

7-Nitro-5-(*N*-oxyaza)-QX (**12e**) has been found to have robust antinociceptive activity in the tail flick test in mice.[37] NMDA receptor antagonists such as ketamine and MK-801 were found to be essentially inactive in this model, while the selective AMPA antagonist NBQX was highly active. Specifically, upon intrathecal administration (*N*-oxyaza)-QX **12e** had an ED_{50} value of 2.6 μg/mouse and NBQX had an ED_{50} value of 1.6 μg/mouse.[37] Since NBQX is much more potent than QX **12e** at AMPA receptors (0.052 μM vs 1.3 μM), these results suggest that, although inhibition of AMPA receptors by **12e** might be the primary mechanism of antinociception in the tail flick test model, inhibition of NMDA receptors by **12e** may augment these effects. Broad spectrum glutamate receptor antagonists might, therefore, be better analgesics than selective AMPA receptor antagonists. Due to their good aqueous solubility, (*N*-oxyaza)-QX **12e** and other (*N*-oxyaza)-QXs in this series have potential utility as injectable analgesics for the treatment of acute or chronic pain.

Lastly, 7-chloro-5-(*N*-oxyaza)-QX (**12a**) has been tested in a rat permanent middle cerebral artery (MCA) occlusion model of focal ischemia and found to provide significant neuroprotection. Administration of QX **12a** as a 20 mg/kg iv bolus followed by 14 mg/kg/h iv infusion for 22 h immediately after MCA occlusion produced a 67% reduction in cortical infarct volume.[38] Thus, (*N*-oxyaza)-QX **12a** is a potential neuroprotectant for the treatment of stroke.

Conclusion

In conclusion, a series of 5-aza-7-substituted-QXs and 5-(*N*-oxyaza)-7-substituted-QXs were synthesized and evaluated as antagonists of NMDA/glycine, AMPA and KA receptors. (*N*-Oxyaza)-QXs **12a**−**e** have low micromolar or submicromolar potency for NMDA/glycine receptors and low micromolar potencies for AMPA and KA receptors. QXs **12a**−**e** are about 2−12-fold less potent at AMPA receptors than at NMDA/glycine receptors and QX **12e** has the closest balance between NMDA/glycine and AMPA receptor potencies (2-fold). (*N*-Oxyaza)-QXs **12a**−**e** all have high *in vivo* potency as anticonvulsants in the MES model. (*N*-Oxyaza)-QXs **12a** and **12e** also have robust effects in neuroprotection and antinociception assays, respectively. In terms of activity *in vivo*, these (*N*-oxyaza)-QXs are among the most potent broad spectrum glutamate antagonists reported to date.

Experimental Section

Chemistry. Melting points were determined in open capillary tubes on a Mel-Temp apparatus and are uncorrected. The ^{1}H NMR spectra were recorded at 300 MHz. Chemical shifts are reported in ppm (δ) and *J* coupling constants are reported in Hz. ^{19}F NMR spectra were recorded with C_6F_6 as internal standard (-162.9 ppm). Elemental analyses were performed by Desert Analytics, Tucson, AZ. Mass spectra (MS) were obtained with a VG 12-250 or a VG ZAB-2FHF mass spectrometer. QXs **13a** and **13e** have been reported previously.[24]

2-Amino-5-chloro-3-nitropyridine (9a). To 100 mL of H_2SO_4 (97%) kept in an ice bath was added portionwise 25.7 g (0.200 mol) of 2-amino-5-chloropyridine (**8a**). The resulting solution was heated to 55 °C and 13.5 mL (0.211 mol) of HNO_3 (d = 1.41, 70%) was added dropwise to keep the temperature at 55−60 °C (taking about 2 h). The solution was heated at 55−60 °C for 1 h after addition of HNO_3. It was poured into 600 mL of ice−water and partially neutralized with 40% NaOH (about 200 mL). The yellow precipitate was filtered, washed by water, and dried to leave 19.5 g (56%) of **9a** as a yellow powder: ^{1}H NMR ($CDCl_3$) 6.70 (mb, 2H), 8.33 (d, J = 2.4, 1H), 8.43 (d, J = 2.4, 1H).

2-Amino-5-methyl-3-nitropyridine (9c). To a stirred solution of 2-amino-5-methylpyridine (**8c**) (2.16 g, 20.0 mmol) in 10 mL of H_2SO_4 was added dropwise HNO_3 (70%, 2.0 mL,

31.5 mmol) at rt. The mixture was then stirred at 55 °C for 2 h, cooled to rt, and poured into crashed ice (about 100 g). The mixture was basified to pH 9 in an ice bath by dropwise addition of 40% aqueous NaOH and the mixture was extracted with $CHCl_3$ (5 × 25 mL). The $CHCl_3$ extracts were combined, washed with brine (20 mL), dried ($MgSO_4$), and rotary evaporated to give 770 mg (25%) of **9c** as a bright yellow powder: mp 175−176 °C; ^{1}H NMR ($CDCl_3$) 2.29 (s, 3H), 6.56 (bs, 2H), 8.22 (s, 1H), 8.24 (s, 1H).

2-Amino-3-nitro-5-(trifluoromethyl)pyridine (9d). To a stirred solution of 2-amino-5-(trifluoromethyl)pyridine (**8d**) (1.70 g, 10.5 mmol) in 10 mL of H_2SO_4 heated at 50 °C was added dropwise HNO_3 (70%, 1.7 mL, 26 mmol) over 10 min. The resulting solution was stirred at 80 °C for 46 h and then allowed to cool to rt, poured into ice−water (100 mL), and basified to pH 9 with 40% aqueous NaOH. The resulting mixture was extracted with ethyl acetate (5 × 40 mL). The extracts were dried ($MgSO_4$) and rotary evaporated to give 1.33 g (61%) of **9d** as a yellow powder: mp 191−193 °C; ^{1}H NMR ($CDCl_3$) 6.14 (bs, 1H), 7.92 (bs, 1H), 8.59 (s, 1H), 8.67 (s, 1H).

2-Amino-3,5-dinitropyridine (9e). To a stirred mixture of 2-chloro-3,5-dinitropyridine (**7**) (2.035 g, 10 mmol) in EtOH (15 mL) was added dropwise aqueous NH_4OH (6 mL) at rt over 20 min. The resulting mixture was stirred at rt for 15 min and then cooled to 0 °C. The precipitate was filtered, washed with water (3 × 5 mL), and dried to give 1.64 g (89%) of **9e** as a yellow powder: mp 190−191 °C; ^{1}H NMR (DMSO-d_6) 8.70 (bs, 1H), 8.95 (d, J = 2.5, 1H), 9.16 (d, J = 2.5, 1H), 9.22 (bs, 1H).

2,3-Diamino-5-chloropyridine (10a). A mixture of **9a** (22.0 g, 127 mmol), Raney Ni (2.2 g), and MeOH (220 mL) was shaken under H_2 (20−40 psi) for 3 h and then filtered. The filtrate was rotary evaporated and the residual solid was dried to give 17.6 g (96%) of **10a** as a tan powder: mp 169−171 °C (lit.[27] mp 172−173 °C); ^{1}H NMR (DMSO-d_6) 4.99 (bs, 2H), 5.56 (bs, 2H), 6.68 (d, J = 2.1, 1H), 7.20 (d, J = 2.1, 1H).

2,3-Diamino-3-bromopyridine (10b). A mixture of 2-amino-5-bromo-3-nitropyridine (**9b**) (676 mg, 3.10 mmol), tin(II) chloride dihydrate (3.58 g, 14.0 mmol), and EtOH (3 mL) was heated to boil. The resulting solution was refluxed under N_2 for 15 h, cooled to rt, and evaporated to dryness. To the residual solid was added H_2O (80 mL), and the mixture was basified to pH 8 with 1 N aqueous NaOH. The resulting mixture was extracted with ethyl acetate (3 × 50 mL). The extracts were combined, washed with brine (25 mL), dried ($MgSO_4$), and rotary evaporated to dryness. The residual solid was dried at 40 °C under vacuum, giving 565 mg (97%) of **10b** as a pale yellow powder: mp 158−160 °C; ^{1}H NMR ($CDCl_3$ + DMSO-d_6) 3.82 (s, 2H), 4.53 (s, 2H), 6.84 (s, 1H), 7.45 (s, 1H).

2,3-Diamino-5-methylpyridine (10c). A mixture of nitropyridine **9c** (790 mg, 5.17 mmol), methanol (60 mL) and 5% Pd−C (70 mg) was shaken under H_2 (20−30 psi) for 3 h and then filtered and evaporated to give 629 mg (99%) of the diamine **10c** as a thick black oil: ^{1}H NMR ($CDCl_3$) 2.16 (s, 3H), 3.31 (bs, 2H), 4.16 (bs, 2H), 6.74 (s, 1H), 7.46 (s, 1H).

2,3-Diamino-5-(trifluoromethyl)pyridine (10d). A mixture of **9d** (950 mg, 4.59 mmol), methanol (15 mL), and Raney Ni (200 mg) was shaken under H_2 (30−40 psi) for 2 h and then filtered. The filtrate was evaporated to dryness, giving 810 mg (100%) of the diamine **10d** as a deep yellow powder: mp 97−99 °C; ^{1}H NMR ($CDCl_3$) 3.39 (bs, 2H), 4.56 (bs, 2H), 7.05 (s, 1H), 7.93 (s, 1H).

2,3-Diamino-5-nitropyridine (10e). To a stirred suspension of **9e** (1.62 g, 8.79 mmol) in MeOH (75 mL) was added dropwise 20% aqueous $(NH_4)_2S$ (15 mL, 44 mmol) at rt. The resulting dark-red solution was stirred at rt for 0.5 h, refluxed for 0.5 h, and then cooled to rt. The resulting mixture was filtered. The filtrate was concentrated to about 30 mL and cooled in ice−water. The precipitate was filtered, washed with cooled EtOH (2 × 5 mL), and dried to give 1.09 g (80%) of **10e** as a deep red powder: mp 260−262 °C (dec); ^{1}H NMR (DMSO-d_6) 5.31 (s, 2H), 6.98 (s, 2H), 7.34 (d, J = 2.2, 1H), 8.17 (d, J = 2.2, 1H).

5-Aza-7-chloro-1,4-dihydroquinoxaline-2,3-dione (11a). A solution of the diamine **10a** (17.57 g, 122.4 mmol) and oxalic acid (13.22 g, 146.9 mmol) in 2 N aqueous HCl (160 mL) was refluxed under N_2 for 16 h and then cooled to rt. The precipitate was filtered, washed with water (4 × 15 mL), and dried to give 20.26 g (84%) of **11a** as a dark brown powder: mp >360 °C; ^{1}H NMR (DMSO-d_6) 7.44 (d, J = 2.1, 1H), 8.10 (d, J = 2.1, 1H), 12.04 (bs, 1H), 12.48 (bs, 1H); HRMS calcd for $C_7H_4{}^{35}ClN_3O_2$ 196.9988, found 196.9993. Anal. ($C_7H_4ClN_3O_2 \cdot 0.25H_2O$) C: calcd, 41.60; found, 41.15. H: calcd, 2.24; found 1.75. N.

5-Aza-7-bromo-1,4-dihydroquinoxaline-2,3-dione (11b) was prepared similarly to **11a**. From a solution of diamine **10b** (500 mg, 2.66 mmol) and oxalic acid (360 mg, 4.00 mmol) in 2N aqueous HCl (3.0 mL) there was obtained 536 mg (83%) of **11b** as a green-yellow powder: mp >370 °C; ^{1}H NMR (DMSO-d_6) 7.55 (s, 1H), 8.16 (s, 1H), 12.01 (s, 1H), 12.46 (s, 1H). Anal. ($C_7H_4BrN_3O_2$) C, H, N.

5-Aza-7-methyl-1,4-dihydroquinoxaline-2,3-dione (11c) was prepared similarly to **11a**. From a solution of diamine **10c** (625 mg, 5.08 mmol) and oxalic acid (685 mg, 7.61 mmol) in 2N aqueous HCl (10 mL) there was obtained 393 mg (44%) of **11c** as a black powder: mp >370 °C (lit.[12] mp >300 °C); ^{1}H NMR (DMSO-d_6) 2.26 (s, 3H), 7.25 (s, 1H), 7.90 (s, 1H), 11.95 (s, 1H), 12.24 (s, 1H). Anal. ($C_8H_7N_3O_2 \cdot 0.5\ H_2O$) C, H, N.

5-Aza-7-(trifluoromethyl)-1,4-dihydroquinoxaline-2,3-dione (11d). A stirred mixture of diamine **10d** (800 mg, 4.52 mmol) and diethyl oxalate (7.00 g, 47.9 mmol) was heated at 160 °C for 2 h and cooled to rt. The mixture was diluted with hexane (20 mL), filtered, washed with hexane (3 × 5 mL), and dried to give 997 mg (94%) of **11d** as a yellow powder: mp >360 °C; ^{1}H NMR (DMSO-d_6) 7.63 (s, 1H), 8.44 (s, 1H), 12.12 (s, 1H), 12.70 (s, 1H); ^{19}F NMR (DMSO-d_6) −131.2 ppm. Anal. ($C_8H_4F_3N_3O_2$) C, H, N.

5-Aza-7-nitro-1,4-dihydroquinoxaline-2,3-dione (11e) was prepared similarly to **11a**. From a solution of diamine **10e** (308 mg, 2.00 mmol) and oxalic acid (270 mg, 3.00 mmol) in 2 N aqueous HCl (3 mL) there was obtained 308 mg (74%) of **11e** as a black powder: mp >370 °C (lit.[12] mp >300 °C); ^{1}H NMR (DMSO-d_6) 8.09 (d, J = 2.4, 1H), 8.91 (d, J = 2.4, 1H), 12.22 (bs, 2H); HRMS calcd for $C_7H_4N_4O_4$ 208.231, found 208.233. Anal. ($C_7H_4N_4O_4 \cdot 0.4H_2O$) C, H, N.

7-Chloro-5-(*N*-oxyaza)-1,4-dihydroquinoxaline-2,3-dione (12a). (a) To a solution of aza-QX **11a** (77 mg, 0.39 mmol) in 5 mL of trifluoroacetic acid (TFA) was added *m*-chloroperbenzoic acid (MCPBA, 280 mg, 0.8 mmol) at rt. The resulting red solution was refluxed for 15 h and then rotary evaporated to dryness. The residual solid was washed with MeOH (3 × 5 mL) and dried to give 80 mg (96%) of **12a** as an off-white powder: mp 321−323 °C; ^{1}H NMR (DMSO-d_6) 7.05 (s, 1H), 8.39 (s, 1H), 12.20 (bs, 2H).

(b) To a stirred solution of 30% H_2O_2 (260 mL) was added dropwise trifluoroacetic anhydride (130 mL) at 0 °C over 1 h. After the addition, the resulting solution was stirred at 0 °C for 30 min, then the aza-QX **11a** (25.68 g, 130 mmol) was added in one portion. The mixture was stirred at 90 °C for 30 min (a lot of gas evolved). The resulting orange solution was cooled in an ice bath and stirred for 1 h. The precipitate was filtered, washed with MeOH (2 × 25 mL) and H_2O (2 × 25 mL), and dried to give 25.86 g (93%) of **12a** as a yellowish powder: HRMS calcd for $C_7H_4{}^{35}ClN_3O_3$ 212.9939, found 212.9953. Anal. ($C_7H_4ClN_3O_3 \cdot H_2O$) C, H, N.

7-Bromo-5-(*N*-oxyaza)-1,4-dihydroquinoxaline-2,3-dione (12b) was prepared similarly to **12a** (method a). From a mixture of **11b** (135 mg, 0.558 mmol), MCPBA (266 mg, 0.76 mmol), and TFA (3.0 mL) there was obtained 65 mg (45%) of **12b** as a yellowish powder: mp 304−306 °C (dec); ^{1}H NMR (DMSO-d_6) 7.12 (s, 1H), 8.48 (s, 1H), 12.17 (bs, 2H). Anal. ($C_7H_4BrN_3O_3 \cdot 1.05H_2O$) C, H, N.

7-Methyl-5-(*N*-oxyaza)-1,4-dihydroquinoxaline-2,3-dione (12c) was prepared similarly to **12a** (method a). From a mixture of **11c** (177 mg, 1.0 mmol), MCPBA (542 mg, 1.5 mmol), and TFA (5.0 mL) there was obtained 135 mg (74%) of **12c** as a yellow powder: mp 228−230 °C (dec); ^{1}H NMR (DMSO-d_6) 2.22 (s, 3H), 6.85 (s, 1H), 7.99 (s, 1H), 12.12 (s, 2H). Anal. ($C_8H_7ClN_3O_3 \cdot 1.25H_2O$) C, H, N.

5-(*N*-Oxyaza)-7-(trifluoromethyl)-1,4-dihydroquinoxaline-2,3-dione (12d). To 30% H_2O_2 (4 mL) was added trifluoroacetic anhydride (2 mL) at 0 °C dropwise with stirring.

The resulting solution was stirred at 0 °C for 30 min, then aza-QX **11d** (462 mg, 2.0 mmol) was added in one portion. The mixture was stirred at 90 °C for 1 h. The resulting solution was evaporated and the residue was coevaporated with EtOH (5 × 5 mL). The residual solid was mixed with EtOH (20 mL), filtered, washed with EtOH (2 × 5 mL), and dried to give 338 mg (68%) of **12d** as an off-white powder: mp 329−330 °C (dec); ^{1}H NMR (DMSO-d_6) 7.20 (s, 1H), 8.66 (s, 1H), 12.31 (bs, 2H); ^{19}F NMR (DMSO-d_6) −131.9 ppm; HRMS calcd for $C_8H_4F_3N_3O_3$ 247.0203, found 247.0204. Anal. ($C_8H_4F_3N_3O_3$·0.2 H_2O) C, H, N.

7-Nitro-5-(*N*-oxyaza)-1,4-dihydroquinoxaline-2,3-dione (12e). To 30% H_2O_2 (2 mL) was added trifluoroacetic anhydride (1 mL) at 0 °C dropwise with stirring. After the addition, the resulting solution was stirred at rt for 30 min, then aza-QX **11e** (104 mg, 0.5 mmol) was added. The black suspension was stirred at 90 °C for 0.5 h and cooled to rt, and EtOH (5 mL) was added. The precipitate was filtered, washed with EtOH (2 × 1 mL), and dried to give 86 mg (76%) of **12e** as a yellow powder: mp 325 °C (dec); ^{1}H NMR (DMSO-d_6) 7.73 (d, J = 2.1, 1H), 8.95 (d, J = 2.1, 1H), 12.38 (bs, 2H); HRMS calcd for $C_7H_4N_4O_5$ 224.0180, found 224.0195; Anal. ($C_7H_4N_4O_5$·H_2O) C, H, N.

Pharmacology. DCKA Binding Assay. The potency of the QXs at the glycine site of the NMDA receptor was determined by inhibition of [^{3}H]DCKA binding in rat brain cortical membranes as previously described.[28] Briefly, well-washed membranes (400 μg) were incubated in 50 mM HEPES−KOH (pH 7.5) with 15 nM [^{3}H]DCKA for 30 min at 0 °C. Nonspecific binding was defined using ACEA 1021 (10 μM). Assays were terminated by filtration on to Schleicher and Schuell No. 32 filters using a precooled Brandel harvester and counted using a Beckman LS-6000 scintillation counter.

AMPA Binding Assay. The potency of the QXs at the agonist recognition site of the AMPA receptor was determined by inhibition of [^{3}H]-(*S*)-AMPA binding in whole rat brain membranes as previously described.[29] Briefly, well-washed, Triton X-100-treated membranes (200 μg) were incubated in 50 μM EGTA/50 mM KSCN/100 mM Tris-acetate (pH 7.2) with 10 nM [^{3}H]-(*S*)-AMPA for 60 min. Nonspecific binding was defined using glutamate (1 mM). Assays were terminated by filtration onto GF/B filter plates using a Packard FilterMate 96-well plate harvester and counted using a Packard TopCount scintillation counter.

KA Binding Assay. The potency of the QXs at the agonist recognition site of the high-affinity KA receptor was determined by inhibition of [^{3}H]KA binding in whole rat brain membranes according to the method of Johansen *et al*.[30] with modifications. Briefly, well-washed, Triton X-100-treated whole rat brain membranes were prepared as for the AMPA assay,[29] except that the final three wash steps were done using 50 mM Tris-HCl (pH 7.1) buffer. Membranes (200 μg) were incubated in 50 mM Tris-HCl (pH 7.1) with 2 nM [^{3}H]kainate for 60 min at 0 °C. Nonspecific binding was defined using glutamate (1 mM). Assays were terminated as in the AMPA assay.

Data Analysis. IC_{50} values were determined using the sigmoidal equation in Prism (GraphPad) from nine point concentration−response curves (test compound added in duplicate in 5 μL of DMSO; 1% final).

Electrophysiology. Solutions of QXs were made up in DMSO. Ringer solutions were made by 300−1000-fold dilution of DMSO stocks. Agonist concentration−response curves were analyzed as described previously.[25] For NR1A/2C receptors the EC_{50} value for glycine was 0.17 μM and the slope was 1.5 (n = 6). For AMPA receptors expressed by rat cerebral cortex poly(A^+) RNA, the EC_{50} value was 5.9 μM and the slope was 2.0 (n = 5). K_b values in Table 2 were calculated from three or four point concentration−inhibition curves using the equation:

$$K_b = \frac{IC_{50}}{\{2 + ([\text{agonist}]_f/EC_{50})^n\}^{1/n} - 1}$$

where IC_{50} is the concentration of QX that reduces the control response by 50%, $[\text{agonist}]_f$ is the fixed dose of agonist used to construct the inhibition curve, EC_{50} is the concentration of agonist evoking a half-maximal response, and n is the slope of the agonist concentration−response relation.[39]

Mouse Maximum Electroshock-Induced Seizure Tests. Procedures for the mouse MES assay were as reported previously.[32,33] QXs **11e** and **12a**−**e** were dissolved in 0.05 M Tris and were tested for anticonvulsant effects 2 min after iv administration (the peak of activity). ED_{50} values were determined by Litchfield and Wilcoxon analysis of three independent experiments.

Acknowledgment. We thank Dr. Yan Ni and Dr. Ricardo Miledi (University of California, Irvine) for the generous gift of rat cerebral cortex poly(A)$^+$ RNA used in this study, Dr. P. H. Seeburg (Heidelburg University, Heidelburg, Germany) for the cDNAs encoding rat NR1 and NR2 subunits, Dr. John Guastella (CoCensys Inc.) for preparation of cRNAs encoding NMDA receptor subunits, and Mr. Joe Richman (CoCensys, Inc.) for measurements of log *P*. Financial support was provided in part by CoCensys, Inc., and the National Institute of Drug Abuse (DA-06726).

References

(1) (a) Monaghan, D. T.; Bridges, R. J.; Cotman, C. W. The Excitatory Amino Acid Receptors: Their Classes, Pharmacology, and Distinct properties in the Function of the Central Nervous System. *Annu. Rev. Pharmacol. Toxicol.* **1989**, *29*, 365−402. (b) Johnson, R. L.; Koerner, J. F. Excitatory Amino Acid Neurotransmission. *J. Med. Chem.* **1988**, *31*, 2057−2066.

(2) Gasic, G. P.; Hollmann, M. Molecular Neurobiology of Glutamate Receptors. *Annu. Rev. Physiol.* **1992**, *54*, 507−36.

(3) Seeberg, P. H. The Molecular Biology of Mammalian Glutamate Receptor Channels. *Annu. Rev. Neurosci.* **1993**, *16*, 359−365.

(4) Knopfel T.; Kuhn, R.; Allgeier, H. Metabotropic Glutamate Receptors: Novel Targets for Drug Development. *J. Med. Chem.* **1995**, *38*, 1417−1426.

(5) Meldrum, B.; Garthwaite, J. Excitatory Amino Acid Neurotoxicity and Neurodegenerative Disease. *Trends Pharmacol. Sci.* **1990**, *11*, 379−387.

(6) Lipton, S. T.; Rosenberg, P. A. Excitatory Amino Acids as a Final Common Pathway for Neurologic Disorders. *New. Eng. J. Med.* **1994**, *330*, 613−622.

(7) Doble, A. Excitatory Amino Acid Receptors and Neurodegeneration. *Therapie* **1995**, *50*, 319−337.

(8) Muir, K. W.; Lees, K. R. Clinical Experience with Excitatory Amino Acid Antagonist Drugs. *Stroke* **1995**, *26*, 503−513.

(9) (a) Wong, E. H. F.; Kemp, J. A. Sites for Antagonism on the *N*-Methyl-D-aspartate Receptor Channel Complex. *Annu. Rev. Pharmacol. Toxicol.* **1991**, *31*, 401−425. (b) Bigge, C. F. Structural Requirements for the Development of Potent *N*-Methyl-D-aspartic Acid Receptor Antagonists. *Biochem. Pharmacol.* **1993**, *45*, 1547−1561. (c) Leeson, P. D.; Iversen, L. L. The Glycine Site on the NMDA Receptor: Structure−Activity Relationships and Therapeutic Potential. *J. Med. Chem.* **1994**, *37*, 4053−4067.

(10) Bigge, C. F.; Boxer, P. A.; Ortwine, D. F. AMPA/Kainate Receptors. *Current Pharm. Design* **1996**, *2*, 397−412.

(11) (a) Sheardown, M. J.; Nielsen, E. Q.; Hansen, A. J.; Jacobsen, P.; Honore, T. 2.3-Dihydroxy-6-nitro-7-sulfamoyl-benzo(F)quinoxaline: A Neuroprotectant for Cerebral Ischemia. *Science* **1990**, *247*, 571−574. (b) Ohmori, J.; Sakamoto, S.; Kubota, H.; Shimizu-sasamata, M.; Okada, M.; Kawasaki, S.; Hidaka, K.; Togami, J.; Kuruya, T.; Murase, K. 6-(1*H*-Imidazol-1-yl)-7-nitro-2,3(1*H*,4*H*)-quinoxalinedione Hydrochloride (YM90K) and Related Compounds: Structure-Activity Relationships for the AMPA-Type Non-NMDA Receptor. *J. Med. Chem.* **1994**, *37*, 467−475.

(12) Ohmori, J.; Kubota, H.; Shimizu-Sasamata, M.; Okada, M.; Sakamoto, S. Novel α-Amino-3-hydroxy-5-methylisoxazole-4-propionate Receptor Antagonists: Synthesis and Structure-Activity Relationships of 6-(1*H*-Imidazol-1-yl)-7-nitro-2,3-(1*H*,4*H*)-pyrido[2,3-*b*]pyrazinedione and Related Compounds. *J. Med. Chem.* **1996**, *39*, 1331−1338.

(13) (a) Ornstein, P. L.; Arnold, M. B.; Augenstein, N. K.; Lodge, D.; Leander, J. D.; Schoepp, D. D. (3*SR*,4a*RS*,6*RS*,8a*RS*)-6-[2-(1H-Tetrazol-5-yl)ethyl]decahydroisoquinoline-3-carboxalic Acid: A structurally Novel, Systemically Active, Competitive AMPA Receptor Antagonist. *J. Med. Chem.* **1993**, *36*, 2046−2048. (b) Ornstein, P. L.; Arnold, M. B.; Allen, N. K.; Bleisch, T.; Borromeo, P. S.; Lugar, C. W.; Leander, J. D.; Lodge, D.;

Schoepp, D. D. Structure−Activity Studies of 6-(Tetrazolylalkyl)-Substituted Decahydroisoquinoline-3-carboxylic Acid AMPA Receptor Antagonists. 1. Effects of Stereochemistry, Chain Length, and Chain Substitution. *J. Med. Chem.* **1996**, *39*, 2219−2231. (c) Ornstein, P. L.; Arnold, M. B.; Allen, N. K.; Bleisch, T.; Borromeo, P. S.; Lugar, C. W.; Leander, J. D.; Lodge, D.; Schoepp, D. D. Structure−Activity Studies of 6- Substituted Decahydroisoquinoline-3-carboxylic Acid AMPA Receptor Antagonists. 2. Effects of Distal Acid Bioisosteric Substitution, Absolute Stereochemical Preferences, and in Vivo Activity. *J. Med. Chem.* **1996**, *39*, 2232−2244.

(14) (a) Tarnawa, I.; Berzsenyi, P.; Andrasi, F.; Botka, P.; Hamoni, T.; Ling, I.; Korosi, J. Structure-Activity Relationships of 2,3-Benzodiazepine Compounds with Glutamate Antagonistic Action. *Bio. Med. Chem. Lett.* **1993**, *3*, 99−104. (b) Rogawski, M. A. Therapeutic Potential of Excitatory Amino Acid Antagonists: Channel Blockers and 2,3-Benzodiazepines. *Trends Pharmacol. Sci.* **1993**, *14*, 325−331. (c) Vizi, E. S.; Mike, A.; Tarnawa, I. 2,3-Benzodiazepines (GYKI 52466 and Analogs): Negative Allosteric Modulators of AMPA Receptors. *CNS Drug Rev.* **1996**, *2*, 91−126.

(15) Anderson, B. A.; Hansen, M. M.; Harkness, A. R.; Henry, C. L.; Vicenzi, J. T.; Zmijewski, M. J. Application of a Practical Biocatalytic Reduction to an Enantioselective Synthesis of the 5*H*-2,3-Benzodiazepine LY300164. *J. Am. Chem. Soc.* **1995**, *117*, 12358−12359.

(16) Thomas, R. J. Excitatory Amino Acids in Health and Disease. *J. Am. Geriatrics Soc.* **1995**, *43*, 1279−89.

(17) Czuczwar, S. J.; Borowicz, K. K.; Kleinrok, Z.; Tutka, P.; Zarnowski, T.; Turski, W. A. Influence of Combined Treatment with NMDA and Non-NMDA Receptor Antagonists on Electroconvulsions in Mice. *Eur. J. Pharmocol.* **1995**, *281*, 327−333.

(18) Zhou S.; Bonasera, L.; Carlton, S. M. Peripheral Administration of NMDA, AMPA or KA Results in Pain Behaviors in Rats. *NeuroReport* **1996**, *7*, 895−900.

(19) Buchan, A. M.; Lesiuk, H.; Barnes, K. A.; Li, H.; Huang, Z.-G.; Smith, K. E.; Xue, D. AMPA Antagonists: Do They Hold More Promise for Clinical Stroke Trials Than NMDA Antagonists? *Stroke, Suppl I.* **1993**, *24*, I-148−152.

(20) Carling, R. W.; Leeson, P. D.; Moore, K. W.; Smith, J. D.; Moyes, C. R.; Mawer, I. M.; Thomas, S.; Chan, T.; Baker, R.; Foster, A. C.; Grimwood, S.; Kemp, J. A.; Marshall, G. R.; Tricklebank, M. D.; Saywell, Kay L. 3-Nitro-3,4-dihydro-2(1*H*)-quinolones. Excitatory Amino Acid Antagonists Acting at Glycine-Site NMDA and (RS)-α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic Acid Receptors. *J. Med. Chem.* **1993**, *36*, 3397−3408.

(21) Bigge, C. F.; Malone, T. C.; Boxer, P. A.; Nelson, C. B.; Ortwine, D. F.; Schelkun, R. M.; Retz, D. M.; Lescosky, L. J.; Borosky, S. A.; Vartanian, M. G.; Schwarz, R. D.; Campbell, G. W.; Robichaud, L. J.; Watjen, F. Synthesis of 1,4,7,8,9,10-Hexahydro-9-methyl-6-nitropyrido[3,4-*f*]-quinoxaline-2,3-dione and Related Quinoxalinediones: Characterization of α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic Acid (and *N*-Methyl-D-aspartate) Receptor and Anticonvulsant Activity. *J. Med. Chem.* **1995**, *38*, 3720−3740.

(22) Ornstein, P. L.; Arnold, M. B.; Allen, N. K.; Leander, J. D.; Tizzano, J. P.; Lodge, D.; Schoepp, D. D. (3*SR*,4a*RS*,6*SR*,8a*RS*)-6-(1*H*-Tetrazol-5-yl)decahydroisoquinoline-3-carboxalic Acid, a Novel, Competitive, Systemically Active NMDA and AMPA Receptor Antagonist. *J. Med. Chem.* **1995**, *38*, 4885−4890.

(23) Cordi, A. A.; Desos, P.; Randel, J. C. R.; Lepagnol, J. Structure-Activity Relationships in a Series of 3-Sulfonylamino-2(1*H*)-quinolones, as New AMPA/Kainate and Glycine Antagonists. *Bio. Med. Chem.* **1995**, *3*, 129−141.

(24) Keana, J. F. W.; Kher, S. M.; Cai, S. X.; Dinsmore, C. M.; Glenn, A. G.; Guastella, J.; Huang, J. C.; Lu, Y.; Ilyin, V.; Lu, Y.; Mouser, P. L.; Woodward, R. M.; Weber, E. Synthesis and Structure-Activity Relationships of Substituted 1,4-Dihydroquinoxaline-2,3-diones (QXs): Antagonists of the Glycine Site on the NMDA Receptor and of Non-NMDA Glutamate Receptors. *J. Med. Chem.* **1995**, *38*, 4367−4379.

(25) Woodward, R. M.; Huettner, J. E.; Guastella, J.; Keana, J. F. W.; Weber, E. *In Vitro* pharmacology of ACEA-1021 and ACEA-1031: Systemically Active quinoxalinediones with High Affinity and Selectivity for *N*-Methyl-D-aspartate Receptor Glycine Sites. *Mol. Pharmacol.* **1995**, *47*, 568−581.

(26) Tran, M.; Lutfy, K.; Xu, Z.; Cai, S. X.; Keana, J. F. W.; Weber, E. The Anticonvulsant Structure-Activity Relationship of a Series of Novel NMDA Receptor/Glycine Site Antagonists in the Maximal Electroshock Seizure (MES) Model in Mice. Presented at 25th Annual Meeting of Society for Neuroscience, San Diego, CA. November, 1995. Abstract 84.18.

(27) Israel, M.; Day, A. R. Preparation of Pyrido-(2,3)-pyrazines, Pyrido-(3,4)-pyrazines and imidazo-(b)-pyridines. *J. Org. Chem.* **1959**, *24*, 1455−1460.

(28) Cai, S. X.; Zhou, Z.-L.; Huang, J.-C; Whittemore, E. R.; Egbuwoku, Z. O.; Lu, Y. Hawkinson, J. E.; Woodward, R. M.; Weber, E.; Keana, J. F. W. Synthesis and Structure-Activity Relationships of 1,2,3,4-Tetrahydroquinoline-2,3,4-trione 3-Oximes: Novel and Highly Potent Antagonists for the NMDA Receptor Glycine Site. *J. Med. Chem.* **1996**, *39*, 3248−3255.

(29) Hawkinson, J. E.; Espitia, S. A. Effects of Thiocyanate and AMPA Receptor Ligands on (*S*)-5-Fluorowillardiine, (*S*)-AMPA and (*R,S*)-AMPA Binding. *Eur. J. Pharmacol.* **1997**, *329*, 213−221.

(30) Johansen, T. H.; Drejer, J.; Watjen, F.; Nielsen, E. O. A Novel Non-NMDA Receptor Antagonist Shows Selective Displacement of Low-Affinity [^{3}H]Kainate Binding. *Eur. J. Pharm. Molec. Pharmacol. Sect.* **1993**, *246*, 195−204.

(31) Monyer, H. R.; Sprengel, R.; Schoepfer, A.; Herb, M.; Higuchi, H.; Lomeli, N.; Burnashev, B.; Sakmann B.; Seeburg, P. H. Heteromeric NMDA Receptors: Molecular and Functional Distinction of Subtypes. *Science* **1992**, *256*, 1217−1221.

(32) Ilyin, V. I.; Whittemore, E. R.; Tran, M.; Shen, K.-Z.; Cai, S. X.; Kher, S. M.; Keana, J. F. W.; Weber, E.; Woodward, R. M. Pharmacology of ACEA-1416: A Potent Systemically Active *N*-Methyl-D-aspartate Receptor Glycine Site Antagonist. *Eur. J. Pharmacol.* **1996**, *310*, 107−114.

(33) Woodward, R. M.; Huettner, J. E.; Tran, M.; Guastella, J.; Keana, J. F. W.; Weber, E. Pharmacology of 5-Chloro-7-(trifluoromethyl)-1,4-dihydro-2,3-quinoxalinedione: A Novel Systemically Active Ionotropic Glutamate Receptor Antagonist. *J. Pharmacol. Exp. Thera.* **1995**, *275*, 1209−1218.

(34) Cai, S. X.; Kher, S. M.; Zhou, Z.-L.; Ilyin, V. I.; Espitia, S. A.; Tran, M.; Hawkinson, J. E.; Woodward, R. M.; Weber, E.; Keana, J. F. W. Structure−Activity Relationships of Alkyl and Alkoxy Substituted 1,4-Dihydroquinoxaline-2,3-diones: Potent and Systemically Active Antagonists for the Glycine Site of the NMDA Receptor. *J. Med. Chem.* **1997**, *40*, 730−738.

(35) Hawkinson, J. E.; Kimbrough, C. L. Unpublished results. The human plasma protein binding was determined by equilibrium dialysis and quantitated by scintillation counting for QX **6** using ^{14}C-labeled material and by UV for (*N*-oxyaza)-QX **12a**.

(36) Hansch, C.; Bjorkroth, J. P.; Leo, A. J. Hydrophobicity and Central Nervous System Agents: On the Principle of Minimal Hydrophobicity in Drug Design. *Pharm. Sci.* **1987**, *76*, 663−687.

(37) Lutfy, K.; Cai, S. X.; Woodward, R. M.; Weber, E. Antinociceptive Effects on NMDA and Non-NMDA Receptor Antagonists in the Tail Flick Test in Mice. *Pain* **1997**, *70*, 31−40.

(38) Daniell, G.; Marek, P.; Weber, E. Unpublished results.

(39) Leff, P.; Dougall, I. G. Further Concerns over Cheng−Prusoff Analysis. *Trend. Pharmacol. Sci.* **1993**, *14*, 110−112.

JM970396Y