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

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We report on a series of alkyl- and alkoxy-substituted 1,4-dihydroquinoxaline-2,3-diones (QXs), prepared as a continuation of our structure−activity relationship (SAR) study of QXs as antagonists for the glycine site of the *N*-methyl-D-aspartate (NMDA) receptor. The *in vitro* potency of these antagonists was determined by displacement of the glycine site radioligand [^{3}H]-5,7-dichlorokynurenic acid ([^{3}H]DCKA) in rat brain cortical membranes. In general, methyl is a good replacement for chloro or bromo in the 6-position, and alkoxy-substituted QXs have lower potencies than alkyl- or halogen-substituted QXs. Ethyl-substituted QXs are generally less potent than methyl-substituted QXs, especially in the 6-position of 5,6,7-trisubstituted QXs. Fusion of a ring system at the 6,7-positions results in QXs with low potency. Several methyl-substituted QXs are potent glycine site antagonists that have surprisingly high *in vivo* activity in the maximal electroshock (MES) test in mice. Among these, 7-chloro-6-methyl-5-nitro QX (**14g**) (IC_{50} = 5 nM) and 7-bromo-6-methyl-5-nitro QX (**14f**) (IC_{50} = 9 nM) are comparable in potency to 6,7-dichloro-5-nitro QX (**2**) (ACEA 1021) as glycine site antagonists. QX **14g** has an ED_{50} value of 1.2 mg/kg iv in the mouse MES assay. Interestingly, alkyl QXs with log *P* values of 0.5 or less tend to be more bioavailable than QXs with higher log *P* values. QX **14g** has 440-fold selectivity for NMDA vs α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, as determined electrophysiologically under steady-state conditions in oocytes expressing rat cerebral cortex poly(A)$^+$ RNA. Overall, **14g** was found to have the best combination of *in vitro* and *in vivo* potency of all the compounds tested in this and previous studies on the QX series.

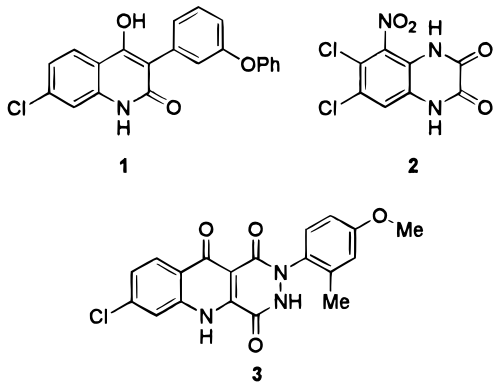
Introduction

Glutamate is the primary excitatory neurotransmitter in the central nervous system. Glutamate receptors are classified into two main groups: ionotropic and metabotropic.[1] The *N*-methyl-D-aspartate (NMDA) type of ionotropic glutamate receptors plays a major role in glutamate excitotoxicity, a process implicated in a number of pathophysiological conditions that lead to neuronal death and degeneration including cerebral ischemia and brain trauma.[2] The discovery that the amino acid glycine is a necessary coagonist for NMDA receptor activation[3] has stimulated research into the development of antagonists for the NMDA receptor glycine site.[4] By inhibiting the glycine site these drugs prevent NMDA receptor activation and hence could potentially be used to reduce damage to the nervous system incurred by cerebral stroke and head injury.

Several structural classes of highly potent glycine site antagonists have been discovered.[5] Many of these compounds, however, have weak *in vivo* activity, probably due to poor penetration of the blood−brain barrier.[6] 4-Hydroxy-3-arylquinolin-2(1*H*)-ones such as **1**,[7] 1,4-dihydroquinoxaline-2,3-diones (QXs) such as **2**,[8] and pyridazino[4,5-*b*]quinolinediones such as **3**[9] are among the few glycine site antagonists shown to be centrally active following systemic administration (Chart 1).

We have recently reported the structure−activity relationship (SAR) of QXs substituted with electron-withdrawing groups such as halogen and nitro.[10] This study led to the discovery of 6,7-dichloro-5-nitro QX (ACEA 1021) (**2**), a potent and systemically active glycine site antagonist[11] currently undergoing clinical evaluation for stroke. As a continuation of SAR studies in the QX series, we elected to explore substitution in the 6- and 7-positions with electron-donating groups such as alkyl, resulting in the discovery of 6,7-dimethyl-5-nitro QX (**9a**) as a potent glycine site antagonist with high *in vivo* activity.[12] Although QX **9a** is about one-fifth as potent as QX **2** at the glycine site, it is slightly more potent *in vivo* as an anticonvulsant in the maximal electroshock (MES) test in mice.[13] On the basis of this observation, we speculated that the combination of alkyl, alkoxy, and halogen substituents in the 6- and 7-positions with a nitro group in the 5-position might

Chart 1



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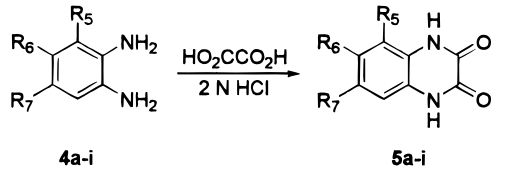
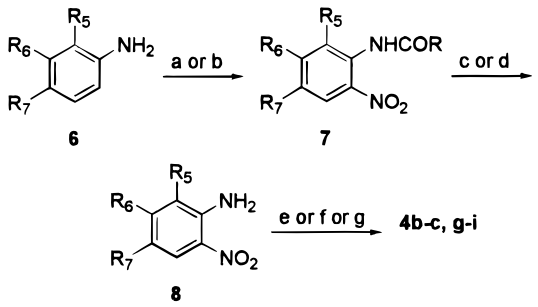
Scheme 1



4a-i 5a-i

a R_5 = H; R_6 = R_7 = Me
b R_5 = H; R_6 = Cl; R_7 = Et
c R_5 = H; R_6 = R_7 = Et
d R_5 = H; R_6 = R_7 = OMe
e R_5 = H; R_6, R_7 = OCH_2O
f R_5 = H; R_6, R_7 = CH=CH-CH=CH
g R_5 = H; R_6, R_7 = $(CH_2)_3$
h R_5 = NO_2; R_6 = H; R_7 = Me
i R_5 = H; R_6 = Br; R_7 = OMe

Scheme 2[a]



6 7 8

[a] (a) (1) $(CH_3CO)_2O$; (2) KNO_3/H_2SO_4; (b) $(CF_3CO)_2O/CF_3CO_2H$, then KNO_3; (c) 2 N HCl; (d) K_2CO_3 or NaOH; (e) $SnCl_2$; (f) $(NH_4)_2S$; (g) H_2/Pd/C.

produce QXs with a favorable combination of *in vitro* and *in vivo* potencies. Herein we report the synthesis and potency of a series of alkyl- and alkoxy-substituted QXs designed to test this hypothesis and the discovery of 7-chloro-6-methyl-5-nitro QX (**14g**), a glycine site antagonist that has the best combination of *in vitro* and *in vivo* activities of all the QXs we have yet tested.[14]

Chemistry

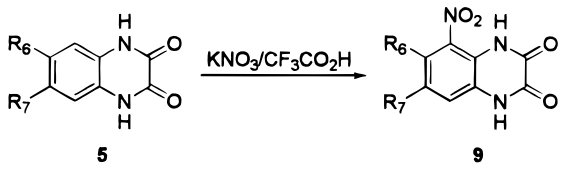
QXs **5a**−**i** were prepared by condensation of oxalic acid with the corresponding 1,2-diaminobenzenes **4a**−**i** (Scheme 1). 6,7-Dihydroxy QX (**5j**) was obtained by demethylation of 6,7-dimethoxy QX (**5d**).

Those 1,2-diaminobenzenes that were not commercially available were prepared from the corresponding anilines using known procedures.[10] Briefly, the amino group of the anilines was first protected by reaction with acetic anhydride to give the amides, which were then nitrated in H_2SO_4 with KNO_3. Alternatively, the aniline was treated with trifluoroacetic anhydride, and then KNO_3 was added to the reaction mixture to give the nitro product. The amides were then hydrolyzed to give the *o*-nitroanilines which were then reduced to give the 1,2-diaminobenzenes (Scheme 2).

3-Chloro-4-ethylaniline (**6b**) was prepared from 2-ethylaniline according to Lambooy and Lambooy.[15] 1,2-Diamino-4,5-diethylbenzene (**4c**) was prepared from 1,2-diethylbenzene using a modified procedure of Lambooy.[16] 1,2-Diamino-5-methyl-3-nitrobenzene (**4h**) was obtained by selectively reducing one of the nitro groups in 4-methyl-2,6-dinitroaniline using $(NH_4)_2S$. 1,2-Diamino-4,5-(methylenedioxy)benzene (**4e**) was prepared by hydrogenation of 4,5-(methylenedioxy)-1,2-dinitrobenzene.[17]

6,7-Disubstituted QXs (**5a**−**c,g**) were treated in concentrated H_2SO_4 or trifluoroacetic acid with KNO_3 to give 5-nitro-6,7-disubstituted QXs (Scheme 3). It was found that KNO_3/CF_3CO_2H is the better nitration system for alkyl QXs. For instance, nitration of **5g** using KNO_3/H_2SO_4 gave a complicated mixture, while mild nitration using KNO_3/CF_3CO_2H gave nitro **9g** as the sole product. Similarly, nitration of 6,7-dimethyl QX (**5a**) by KNO_3/CF_3CO_2H gave a purer product **9a** than that using KNO_3/H_2SO_4. However, when 6-chloro-7-ethyl QX (**5b**) was nitrated, a 1:1 mixture of 5- and 8-nitro isomers was obtained.

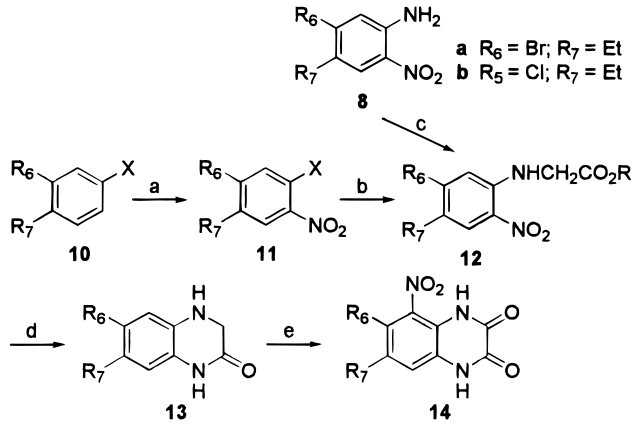
Scheme 3



5 9

a R_6 = R_7 = Me
b R_6 = Cl, R_7 = Et and 8-nitro isomer
c R_6 = R_7 = Et
g R_6, R_7 = $(CH_2)_3$
i R_6 = OMe, R_7 = Br

Scheme 4[a]



8 10 11 12 13 14

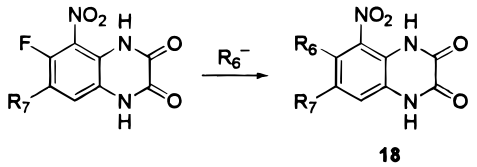
a R_6 = Br; R_7 = Et
b R_6 = Cl; R_7 = Et
c R_6 = Et; R_7 = Cl
d R_6 = Me; R_7 = CN
e R_6 = Me; R_7 = F
f R_6 = Me; R_7 = Br
g R_6 = Me; R_7 = Cl
h R_6= Cl; R_7 = Me
i R_6 = H; R_7 = CN

[a] (a) KNO_3/H_2SO_4; (b) $NH_2CH_2CO_2Na$; (c) $BrCH_2CO_2Et$; (d) $SnCl_2$/EtOH or $Na_2S_2O_4$; (e) HNO_3/CF_3CO_2H.

5-Nitro-6,7-disubstituted QXs **14a**−**h** were prepared by a regiospecific oxidative nitration procedure and the structures were assigned as shown based on our earlier observations (Scheme 4).[18] The intermediate *N*-phenylglycinates (**12**) were prepared by nucleophilic aromatic substitution of 4,5-disubstituted-2-halonitrobenzenes (**11**) using sodium glycinate. Alternatively, nucleophilic substitution on ethyl bromoacetate by 4,5-disubstituted 2-nitroanilines (**8a,b**) gave the *N*-phenylglycinates. The nitro group was reduced by $SnCl_2$ or $Na_2S_2O_4$ and the product spontaneously cyclized to give the 3,4-dihydroquinoxalin-2(1*H*)-ones (**13**). Nitration of the quinoxalin-2-ones with fuming HNO_3 in trifluoroacetic acid resulted in both nitration and oxidation to give 5-nitro-6,7-disubstituted QXs (**14a**−**h**) regiospecifically. Similarly, 4-chloro-3-nitrobenzonitrile was reacted with sodium glycinate followed by reductive cyclization to give 7-cyanoquinoxalin-2-one. Oxidative nitration of the quinoxalin-2-one gave 7-cyano-5-nitro QX (**14i**) regiospecifically.

The 4,5-disubstituted-2-halonitrobenzenes (**11**) were prepared by nitration of 3,4-disubstituted halobenzenes (**10**). 2,5-Dichloro-1-ethylbenzene (**10c**) was prepared

Scheme 5



16 R_7 = F
17 R_7 = Cl

18
a $R_6 = NH_2$; R_7 = F
b R_6 = OMe; R_7 = F
c R_6 = OEt; R_7 = F
d R_6 = OBu-n; R_7 = F
e $R_6 = O(CH_2)_3Ph$; R_7 = F
f R_6 = OMe; R_7 = Cl
g R_6 = SEt; R_7 = Cl

by reduction of 2′,5′-dichloroacetophenone. 3-Bromo-4-ethyl-6-nitroaniline (**8a**) was prepared from 2-ethyl-5-nitroaniline in a manner similar to that of 3-chloro-4-ethyl-6-nitroaniline (**8b**).[15]

6-Chloro-7-methyl QX (**5k**) was prepared by oxidation (H_2O_2/AcOH) of 6-chloro-7-methyl-3,4-dihydroquinoxalin-2(1*H*)-one (**13g**). Reduction of 7-chloro-6-methyl-5-nitro QX (**14g**) with $SnCl_2$ gave 5-amino-7-chloro-6-methyl QX (**15**).

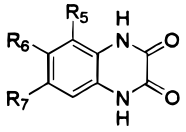
During our formulation and stability experiments with 6-halo-5-nitro-7-substituted QXs, we found that some of these compounds are unstable toward nucleophiles such as amines. In particular, replacement of the fluoro ortho to the nitro group by an amino group was observed. We recognized that the instability of these compounds provides access to a group of 5-nitro-6,7-disubstituted QXs. Thus, nucleophilic substitution of the fluoro ortho to the nitro group in 6,7-difluoro-5-nitro QX (**16**)[10] and 7-chloro-6-fluoro-5-nitro QX (**17**)[10] with different nucleophiles gave QXs **18a**−**g** (Scheme 5). The reaction could be followed by ^{1}H NMR spectroscopy. For QX **16**, replacement of the fluoro ortho to the nitro group by a nucleophile converts the aromatic proton from a doublet of doublets to a doublet. The F−H coupling constants for QXs **18a**−**e** are all around 11.5 Hz, which confirms that the remaining fluoro is ortho to the ring proton.

5-Acetoxy-6,7-dimethoxy QX (**19a**) was obtained by acetoxylation (HNO_3/HOAc) of 6,7-dimethoxy QX (**5d**).[26] Hydrolysis of **19a** gave 5-hydroxy QX (**19b**).

Pharmacology

The potency of QXs for the NMDA receptor glycine site was measured by displacement of [^{3}H]-5,7-dichlorokynurenic acid ([^{3}H]DCKA) binding in rat brain cortical membranes as described previously.[19] For selected QXs, potencies at NMDA receptor glycine sites and at α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors were determined electrophysiologically in *Xenopus* oocytes expressing rat brain poly(A)$^+$ RNA or, in certain cases for NMDA receptor, using the cloned rat NMDA receptor (NR) 1a/2C subunit combinations.[20] 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 and 100 μM glutamate for NMDA receptors; 10 μM AMPA for AMPA receptors.[19] Levels of receptor expression were similar to those reported previously.[10,19] Anticonvulsant activity of selected QXs was measured in a mouse MES model[11,14] which was used as a rough estimate of systemic bioavailability.

Table 1. SAR of 1,4-Dihydroquinoxaline-2,3-diones at the NMDA Receptor Glycine Site



no.	R_5	R_6	R_7	[^{3}H]DCKA IC_{50} (μM)[a]
2	NO_2	Cl	Cl	0.0059 ± 0.0010
5a	H	Me	Me	3.3 ± 0.7
5b	H	Cl	Et	1.4 ± 0.2
5c	H	Et	Et	1.8 ± 0.2
5d	H	OMe	OMe	17 ± 1
5e	H	OCH_2O		>100
5f	H	CH=CHCH=CH		16 ± 1
5g	H	$CH_2CH_2CH_2$		15 ± 4
5h	NO_2	H	Me	9.3 ± 1.9
5i	H	OMe	Br	1.0 ± 0.2
5j	H	OH	OH	>100
5k	H	Cl	Me	0.78 ± 0.06
9a	NO_2	Me	Me	0.029 ± 0.003
9c	NO_2	Et	Et	0.16 ± 0.03
9g	NO_2	$CH_2CH_2CH_2$		3.1 ± 0.5
9i	NO_2	OMe	Br	0.064 ± 0.003
14a	NO_2	Br	Et	0.082 ± 0.019
14b	NO_2	Cl	Et	0.029 ± 0.002
14c	NO_2	Et	Cl	0.13 ± 0.01
14d	NO_2	Me	CN	0.073 ± 0.010
14e	NO_2	Me	F	0.095 ± 0.024
14f	NO_2	Me	Br	0.0087 ± 0.0004
14g	NO_2	Me	Cl	0.0047 ± 0.0006
14h	NO_2	Cl	Me	0.045 ± 0.008
14i	NO_2	H	CN	2.8 ± 0.4
15	NH_2	Me	Cl	0.15 ± 0.03
18a	NO_2	NH_2	F	11 ± 1
18b	NO_2	OMe	F	1.9 ± 0.1
18c	NO_2	OEt	F	3.6 ± 0.7
18d	NO_2	OBu-*n*	F	4.3 ± 0.5
18e	NO_2	$O(CH_2)_3Ph$	F	4.0 ± 0.9
18f	NO_2	OMe	Cl	0.15 ± 0.02
18g	NO_2	SEt	Cl	0.64 ± 0.13
19a	OCOMe	OMe	OMe	53 ± 16
19b	OH	OMe	OMe	17 ± 2
20	H	Cl	Cl	0.13 ± 0.03

[a] Potency is expressed as the concentration producing 50% inhibition (IC_{50}) of [^{3}H]DCKA binding. Values are means ± SEMs of at least three independent experiments.

Results and Discussion

The SAR of QXs as antagonists for the NMDA receptor glycine site is summarized in Table 1. All the 6,7-disubstituted QXs (**5a**−**g,i**−**k**) are less active than 6,7-dichloro QX[10] **20**, implying that electron-withdrawing groups are important for high potency of 6,7-disubstituted QXs. QXs **5b**, **5i**, and **5k**, substituted with one chlorine or bromine atom, are among the most potent inhibitors in this group. 6,7-Dimethyl QX **5a** and 6,7-diethyl QX **5c** have moderate potency, whereas dimethoxy QX **5d**, substituted with two strong electron-donating groups, has low affinity. Dihydroxy QX **5j** is inactive, indicating that hydrophilic groups are not tolerated in the 6- and 7-positions. QX **5e**, with a methylenedioxy ring in the 6,7-position, is inactive, and QXs **5f** and **5g** have low affinity, all suggesting that incorporation of the 6,7-positions into ring systems is not tolerated.

As expected from our earlier SAR of QXs,[10] introduction of a nitro group at the 5-position consistently results in higher affinity. Thus, 6,7-dimethyl-5-nitro QX **9a** is about 100-fold more potent than 6,7-dimethyl QX **5a**, and 6,7-diethyl-5-nitro QX **9c** is about 10-fold more potent than 6,7-diethyl QX **5c**. Likewise, 7-chloro-6-methyl-5-nitro QX **14g** and 6-chloro-7-methyl-5-nitro

Table 2. Functional Antagonism of NMDA and AMPA Receptors Expressed in *Xenopus* Oocytes by QXs

no.	K_b (μM) glycine	K_b (μM) AMPA	selectivity for NMDA[a]	n[b]
9a[c]	0.039 (0.034−0.043)[d]	3.1 (2.7−3.6)	79	4, 4
9g	0.87[e] (0.72−1.0)	17[f] (15−20)	20	3, 3
14d	0.029[e] (0.024−0.035)	0.59[f] (0.51−0.68)	20	3, 4
14g[g]	0.0079 (0.0072−0.0087)	3.5 (3.1−4.0)	440	4, 3

[a] The steady-state selectivity index for inhibition of NMDA receptors was estimated by dividing K_b AMPA by K_b NMDA. [b] Indicates the number of independent experiments (cells examined); numbers refer to NMDA and AMPA, respectively. [c] Data from ref 12b. [d] Numbers in parentheses are 95% confidence intervals adjusted to the linear scale. [e] Apparent antagonist dissociation constants (K_b) for NMDA receptor glycine sites were determined from inhibition of currents activated by 1 μM glycine and 100 μM glutamate in oocytes expressing the cloned rat NMDA receptor subunit combination NR1a/2C. [f] K_b values for AMPA receptors were determined from inhibition of currents activated by 10 μM AMPA in oocytes expressing rat cerebral cortex poly(A)$^+$ RNA. [g] Data from ref 14.

QX **14h** are 160- and 17-fold more potent than 6-chloro-7-methyl QX **5k**, respectively. QX **9g** is the least potent inhibitor among 5-nitro-6,7-disubstituted QXs, again suggesting that incorporation of the 6,7-positions into a ring system considerably reduces potency.

6,7-Dimethyl-5-nitro QX **9a** is >5-fold more potent than 6,7-diethyl-5-nitro QX **9c**, implying the existence of a size-limited pocket in the region surrounding the 6- and 7-positions for 5-nitro-6,7-disubstituted QXs. Since 6-chloro-7-methyl-5-nitro QX **14h** and 6-chloro-7-ethyl-5-nitro QX **14b** have comparable activity while 7-chloro-6-methyl-5-nitro QX **14g** is 26-fold more potent than 7-chloro-6-ethyl-5-nitro QX **14c**, the size-limited effect might be more pronounced at the 6-position compared to the 7-position.

7-Chloro-6-methoxy-5-nitro QX **18f** has affinity comparable to that of 7-chloro-6-ethyl-5-nitro QX **14c**, indicating the similarity of methoxy and ethyl at the 6-position. QX **18f** and QX **14c** is 30 times and 26 times less potent than 7-chloro-6-methyl-5-nitro QX **14g**, respectively, and 7-fluoro-6-methoxy-5-nitro QX **18b** is about 20 times less potent than 7-fluoro-6-methyl-5-nitro QX **14e**. This sharp drop in affinity from methyl to ethyl or methoxy is another indication that there is a size-limited pocket adjacent to the 6-position. Interestingly, QXs **18b**−**e**, with groups ranging in size from methoxy to 3-phenylpropoxy at the 6-position, have comparable affinity, suggesting that after the sharp reduction in potency from methyl to methoxy a further increase in the size of substituents at the 6-position has little effect on potency. Similarly, QX **18g**, with a thioethoxy group at the 6-position, is only 4 times less active than QX **18f**. 6-Amino-7-fluoro-5-nitro QX **18a** is the least potent ligand among QXs **18a**−**e**, again suggesting that hydrophilic groups are not tolerated at the 6-position.

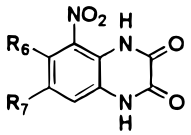
7-Chloro-6-methyl-5-nitro QX **14g** and 7-bromo-6-methyl-5-nitro QX **14f** are the most potent antagonists in this series with IC_{50} values of 5 and 9 nM, respectively. The potency of **14g** is similar to 6,7-dichloro-5-nitro QX **2**, showing that methyl and chloro are comparable at the 6-position. In contrast, 6-chloro-7-methyl-5-nitro QX **14h** is about 7 times less active than QX **2**, indicating that methyl is not such a good replacement for chloro at the 7-position. 7-Fluoro-6-methyl-5-nitro QX **14e** and 7-cyano-6-methyl-5-nitro QX **14d** are about 19 times and 15 times less active than **14g**, respectively, again suggesting that chloro is preferred at the 7-position. Similarly, 7-fluoro-6-methoxy-5-nitro **18b** is about 12 times less potent than 7-chloro-6-methoxy-5-nitro QX **18f**. These observations are in agreement with SAR developed in several other series of glycine antagonists in which a chlorine atom is optimal at the 7-position.[21,22] Interestingly, 6-chloro-7-ethyl-5-nitro QX **14b** is about 5-fold more potent than 7-chloro-6-ethyl-5-nitro QX **14c**, while 7-chloro-6-methyl-5-nitro QX **14g** is about 9-fold more potent than 6-chloro-7-methyl-5-nitro **14h**, indicating that ethyl is better tolerated at the 7-position than at the 6-position, while methyl is better tolerated at the 6-position than at the 7-position.

5-Amino-7-chloro-6-methyl QX **15** is 30 times less potent than the corresponding 5-nitro QX **14g**, indicating the importance of the 5-nitro group. Similarly, 5-acetoxy-6,7-dimethoxy QX **19a** and 5-hydroxy-6,7-dimethoxy QX **19b**[26] have very low potency, again confirming the importance of electron-withdrawing groups at the 5-position. Furthermore, 6,7-dimethyl-5-nitro QX **9a** and 6-chloro-7-methyl-5-nitro QX **14h** are >100-fold more potent than 6,7-dimethyl QX **5a** and 7-methyl-5-nitro QX **5h**, indicating that a substitution pattern encompassing all three positions is critical for high potency of 5-nitro-6,7-disubstituted QXs.[10]

Functional antagonism of NMDA receptor by four selected QXs was tested using electrophysiological recording techniques in *Xenopus* oocytes expressing the cloned rat NMDA receptor subunit combination NR1a/2C, or native rat brain poly(A)$^+$ RNA[8] (Table 2). For QXs **9a** and **14g**, which were measured on rat brain NMDA receptor, the inhibitory potency was similar to that measured in [^{3}H]DCKA binding assay. For QXs **9g** and **14d**, which were measured on NR1a/2C receptor, potency in the electrophysiological assay was ∼3-fold higher than that in the [^{3}H]DCKA binding. This small discrepancy between assays may be due to a slightly higher potency of QXs at NR1a/2C receptors as compared to the mixture of receptors expressed in oocytes by rat whole brain poly(A)$^+$ RNA.[8]

Electrophysiological recordings were also used to test the four selected QXs for functional antagonism of AMPA receptors in oocytes expressing rat cerebral cortex poly(A)$^+$ RNA. Similar to halogen- and nitro-substituted QXs,[10] the alkyl-substituted QXs were all AMPA receptor antagonists (Table 2). 7-Chloro-6-methyl-5-nitro QX **14g** and 6,7-dimethyl-5-nitro QX **9a** have comparable, low micromolar potency for AMPA receptors, but still show >400- and ∼80-fold selectivity for the NMDA glycine site, respectively. These results suggest that, for the AMPA receptors, methyl and chloro are equivalent substituents at the 7-position of QXs. Of the QXs tested, 7-cyano-6-methyl-5-nitro QX **14d** is the most potent AMPA antagonist with a K_b of 590 nM, indicating that for the AMPA receptor, cyano is preferred over methyl and chloro at the 7-position, and this substituent pattern results in a QX which has relatively low selectivity for the glycine site. QX **9g**, with the 6,7-

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



no.	R_6	R_7	[^{3}H]DCKA IC_{50} (nM)	MES ED_{50} mg/kg	log P^a
14g	Me	Cl	4.7	1.2 (0.9−1.6)	0.49
14e	Me	F	95	1.8 (1.3−2.6)	0.08
14h	Cl	Me	45	1.9 (1.2−2.8)	0.44[b]
14f	Me	Br	8.7	2.0 (1.4−2.9)	0.61[b]
9a	Me	Me	29	2.6 (1.8−3.8)	0.58
2	Cl	Cl	5.9	4.0 (3.2−5.0)	0.21
14b	Cl	Et	29	4.5 (3.1−6.4)	0.92[c]
21	Br	Br	20	7.3 (5.7−9.4)	0.74[b]
14a	Br	Et	82	11 (8.5−14)	1.04

[a] Measured by shake-flask method in octanol vs pH 7.4 buffer. [b] Estimated by a HPLC method. [c] Calculated value.

positions cyclized into a ring system, has lower activity at both NMDA and AMPA receptors compared to that of QX **9a**.

To estimate systemic bioavailability, anticonvulsant activity of seven alkyl substituted QXs was measured in a mouse MES model. Table 3 summarizes the anticonvulsant potency and the log *P* values of the seven QXs, together with QX **2** and 6,7-dibromo-5-nitro QX **21,** two of the most potent QXs reported earlier.[10] A surprising number of the alkyl-substituted QXs were potent anticonvulsants following iv administration. Indeed, five of the seven alkyl-substituted QXs tested were more potent anticonvulsants than QX **2**, the most active QX identified in previous studies.[10,11]

The reason for the high *in vivo* activity of these alkyl-substituted QXs remains unclear. As seen in other series of NMDA receptor glycine site antagonists,[6] there is no direct correlation between *in vitro* and *in vivo* activities (Table 3). There is also no direct correlation between *in vivo* activity and log *P* value. Interesting observations can be made, however, by considering the *in vitro, in vivo,* and log *P* data together. For example, QXs **14e** and **14a** have similar *in vitro* potencies, but **14e** has a log *P* value lower than that of **14a** and is >5-fold more active *in vivo.* QXs **9a**, **14b**, and **21**[10] also have similar *in vitro* potencies, but **9a** has a log *P* value lower than that of **14b** and **21**, and **9a** is about 2-fold and 3-fold more active *in vivo* than **14b** and **21,** respectively. In addition, although QX **14e** is 19 times less active than **14g** *in vitro,* it is only <2 times weaker *in vivo.* QXs **14h** and **14f** have log *P* values around 0.5 and are highly active *in vivo.* These data imply that QXs with log *P* values of ∼0.5 or less might be more bioavailable than QXs with higher log *P* values, which is a deviation from the conventional rule of thumb that compounds with a log *P* value around 2 are optimal for CNS drugs.[23] Since QX **2** is highly bound to plasma proteins (>99%),[24] we hypothesize that part of the reason might be that QXs with log *P* values ∼0.5 or less achieve an optimum for limiting binding to plasma proteins and still being sufficiently hydrophobic to penetrate the blood−brain barrier. We speculate that QXs with higher log *P* values might be too tightly bound to plasma proteins, resulting in low *in vivo* activity. Other physiochemical and pharmacokinetic parameters, such as solubility of the QXs in plasma, and clearance rates, are also likely to contribute to the observed difference in *in vivo* potency.

Conclusions

In conclusion, a series of alkyl- and alkoxy-substituted QXs were synthesized and evaluated as antagonists of the NMDA receptor glycine site. Methyl is a good replacement for chloro in the 6-position, and alkoxy-substituted QXs generally have potency lower than alkyl- or halogen-substituted QXs. QXs substituted at the 6-position by groups larger than methyl or chloro show a reduction in potency. 7-Chloro-6-methyl-5-nitro QX (**14g**) and 7-bromo-6-methyl-5-nitro QX (**14f**) are the most active inhibitors with IC_{50} values of 5 and 9 nM in the [^{3}H]DCKA assay, respectively. These two QXs are among the most potent glycine antagonists reported to date. Under steady-state conditions, QX **14g** is >400-fold selective for NMDA receptors vs AMPA receptors as measured electrophysiologically. Most importantly, a number of the alkyl-substituted QXs are potent, systemically active anticonvulsants in the MES assay in mice. QXs **9a** and **14e**−**g** are also neuroprotective in a rat model of focal cerebral ischemia.[25] These compounds therefore have potential as neuroprotectants for the clinical treatment of ischemic stroke.

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 hertz. ^{19}F NMR spectra were recorded with C_6F_6 (−162.9 ppm) as internal standard. Elemental analyses were performed by Desert Analytics, Tucson, AZ. Mass spectra (MS) were obtained using a VG 12-250 or a VG ZAB-2FHF mass spectrometer. Substituted anilines were obtained from Aldrich or Lancaster and used as received. Reagent grade solvents were used without further purification unless otherwise specified. Reverse phase HPLC were obtained at 254 nM on a 4.6 × 250 mM microsorb-MV C18 column, using as solvents 0.1% trifluoroacetic acid in water (A) and 0.1% trifluoroacetic acid in acetonitrile (B). The linear gradient was 20% B in A to 95% B in A with a flow rate of 1 mL/min. The preparation of QXs **5d**, **5i**, **9i**, and **19a,b**[26] and QXs **14e**−**h**[18] have been reported previously.

3-Bromo-4-ethylnitrobenzene. To a mixture of 6.61 g (39.8 mmol) of 2-ethyl-5-nitroaniline[15] in 15 mL of 48% HBr stirred in ice bath was added a solution of 2.84 g (41.1 mmol) of $NaNO_2$ in 5 mL of H_2O. The resulting mixture was stirred in ice bath for 30 min, and then was added in portions into a boiling solution of 3.62 g (25.2 mmol) of CuBr in 8 mL of 48% HBr. The resulting solution was boiled for 1 h, cooled to room temperature, and extracted with $CHCl_3$ (3 × 20 mL). The extract was washed with aqueous 1 N NaOH (10 mL), aqueous 1 N HCl (10 mL), and water (2 × 10 mL), dried ($MgSO_4$), and evaporated to leave 8.5 g (93%) of yellow liquid: ^{1}H NMR ($CDCl_3$) 1.28 (t, J = 7.5, 3H), 2.88 (q, J = 7.5, 2H), 7.40 (d, J = 8.5, 1H), 8.12 (dd, J = 2.3, 8.4, 1H), 8.42 (d, J = 2.2, 1H).

3-Bromo-4-ethylaniline (6a). A solution of 7.8 g (33.9 mmol) of 3-bromo-4-ethylnitrobenzene and 30.6 g (135 mmol) of $SnCl_2 \cdot 2H_2O$ in 75 mL of absolute alcohol was refluxed for 1 h. It was evaporated to remove most of the solvent, and the residue was treated with aqueous 2 N NaOH to pH = 5. The mixture was filtered, and the solid was washed with ethanol (20 mL) and ethyl acetate (40 mL). The filtrate was separated, and the aqueous phase was extracted with ethyl acetate (15 mL). The combined organic phase was dried ($MgSO_4$) and evaporated to leave 6.0 g (88%) of pale-yellow liquid: ^{1}H NMR ($CDCl_3$) 1.17 (t, J = 7.5, 3H), 2.64 (q, J = 7.7, 2H), 3.53 (sb, 2H), 6.58 (dd, J = 2.3, 8.1, 1H), 6.90 (d, J = 2.3, 1H), 7.00 (d, J = 8.1, 1H).

4-Bromo-5-ethyl-2-(trifluoroacetamido)nitrobenzene (7a). To a solution of 30 mL of $(CF_3CO)_2O$ and 30 mL of CF_3CO_2H stirred in ice bath was added dropwise 5.80 g (29.0 mmol) of **6a**, and the resulting mixture was stirred at room temperature for 1 h. To the mixture stirred in ice bath was

added in portion 3.15 g (31.2 mmol) of KNO_3, and it was stirred in an ice bath for 1 h and at room temperature overnight. The solution was added into 150 mL of ice−water, and the precipitate was filtered, washed with water, and dried to give 9.8 g (98%) of pale-yellow solid: ^{1}H NMR ($CDCl_3$) 1.29 (t, J = 7.5, 3H), 2.83 (q, J = 7.4, 2H), 8.16 (s, 1H), 8.99 (s, 1H), 11.30 (s, 1H). The crude product was used for the next reaction without purification.

3-Bromo-4-ethyl-6-nitroaniline (8a). A mixture of 5.10 g (14.7 mmol) of **7a** in 35 mL of 7 wt % K_2CO_3 methanol/H_2O (3:2) was stirred at room temperature for 2 h. It was diluted with 20 mL of water, filtered, washed with water and dried to give 1.81 g (49%) of yellow solid: mp 75−76 °C; ^{1}H NMR ($CDCl_3$) 1.22 (t, J = 7.4, 3H), 2.68 (q, J = 7.6, 2H), 5.93 (br s, 2H), 7.08 (s, 1H), 7.96 (s, 1H).

5-Acetamido-6-nitroindan (7g). To a stirred solution of 5.2 g (39 mmol) of 5-aminoindan (**6g**) in 15 mL of dioxane in an ice bath was added dropwise 8 mL (8.6 g, 84 mmol) of acetic anhydride. The solution was stirred at room temperature for 16 h. It was diluted with 70 mL of water, and the mixture was filtered, washed by water, and dried to give a gray solid: 6.21 g (91%); ^{1}H NMR ($CDCl_3$) 2.07 (m, 2H), 2.17 (s, 3H), 2.88 (m, 4H), 7.20 (mb, 1H), 7.15 (s, 2H), 7.44 (s, 1H).

To a solution of 5.59 g (31.9 mmol) of the amide in 55 mL of H_2SO_4 kept in an ice bath was added portionwise 3.62 g of KNO_3 (35.8 mmol). The solution was stirred in ice bath for 2 h and at room temperature overnight. It was added into 500 mL of ice−water, and the mixture was stirred for 1 h. The precipitate was filtered, washed with water, and dried to leave black solid which was purified by chromatography (silica gel, eluted with hexane/ethyl acetate = 10:1) to give 1.06 g (15%) of a yellow solid: ^{1}H NMR ($CDCl_3$) 2.14 (m, 2H), 2.28 (s, 3H), 3.00 (m, 4H), 8.44 (s, 1H), 8.58 (s, 1H), 10.39 (s, 1H).

5-Amino-6-nitroindan (8g). A mixture of 259 mg (1.16 mmol) of **7g** in 4 mL of 2 N HCl was heated at 85 °C for 9 h and cooled to room temperature. The solid precipitate was filtered, washed with water, and dried to leave 201 mg (97%) of a crystalline yellow solid: ^{1}H NMR ($CDCl_3$) 2.07 (m, 2H), 2.84 (m, 4H), 6.00 (sb, 2H), 6.65 (s, 1H), 7.94 (s, 1H).

5,6-Diaminoindan (4g). A solution of 200 mg (1.12 mmol) of **8g** and 1.14 g (6.01 mmol) of $SnCl_2$ in 8 mL of ethanol was heated at 70 °C for 2 h. It was evaporated to remove the ethanol, and the residue was treated with 40% aqueous NaOH to pH = 12. The mixture was diluted by 4 mL of water and extracted with $CHCl_3$ (3 × 10 mL). The extract was dried ($MgSO_4$) and evaporated to leave 162 mg (97%) of a yellow crystalline solid: ^{1}H NMR ($CDCl_3$) 2.01 (m, 2H), 2.77 (t, J = 7.3, 4H), 3.30 (sb, 4H), 6.61 (s, 2H).

1,2-Diamino-4-chloro-5-ethylbenzene (4b). Diamine **4b** was prepared from **8b**[15] in a manner similar to that for **4g**: ^{1}H NMR ($CDCl_3$) 1.17 (t, J = 7.5, 3H), 2.59 (q, J = 7.4, 2H), 6.56 (s, 1H), 6.70 (s, 1H).

1,2-Diamino-4,5-(methylenedioxy)benzene (4e). 4,5-(Methylenedioxy)-1,2-dinitrobenzene[17] (1.37 g, 6.47 mmol) was dissolved into ethyl acetate (20 mL). To this solution was added 10% Pd/C (343 mg, 20%). The mixture was then stirred at room temperature under the pressure of 40 psi (H_2) for 14 h. The catalyst was removed through a column of Celite (5 g) and washed with ethyl acetate (3 × 15 mL) under nitrogen. The filtrates were combined, and the solvent was removed to give the diamine **4e** as a nearly colorless solid: ^{1}H NMR ($CDCl_3$) 5.80 (s, 2H), 6.34 (s, 2H).

1,2-Diamino-4-methyl-6-nitrobenzene (4h). A dark black solution of 4-methyl-2,6-dinitroaniline (0.100 g, 0.561 mmol) in 6.6% aqueous $(NH_4)_2S$ (3.3 mL) and ethanol (3.5 mL) was refluxed for 45 min. It was then cooled to room temperature, and the volatiles were removed *in vacuo* (inside the hood to avoid the stench of ammonium sulfide). The slurry so obtained was diluted with water (10 mL), and the resulting red solid was filtered and dried under vacuum to give 0.072 g (85%) of **4h** as a red powder which was used as such for the next reaction: ^{1}H NMR (acetone-d_6) 2.21 (s, 3H), 6.34 (br s, 2H), 6.62 (s, 1H), 7.57 (s, 1H).

1,4-Dihydro-6,7-dimethylquinoxaline-2,3-dione (5a). A mixture of 2.72 g (20.0 mmol) of 1,2-diamino-4,5-dimethylbenzene (**4a**) and 1.92 g (21.3 mmol) of oxalic acid in 30 mL of 2 N HCl was refluxed for 2.5 h and cooled to room temperature. The mixture was diluted with 20 mL of H_2O, filtered, washed with water, and dried to give pale-brown solid 3.57 g (94%): mp >250 °C; ^{1}H NMR (DMSO-d_6) 2.16 (s, 6H), 6.87 (s, 2H), 11.79 (s, 2H). Anal. ($C_{10}H_{10}N_2O_2$) C, H, N.

QXs **5b,c,e−h** were prepared from the corresponding 1,2-diaminobenzenes in a manner similar to that for **5a.**

7-Chloro-6-ethyl-1,4-dihydroquinoxaline-2,3-dione (5b): mp >400 °C; ^{1}H NMR (DMSO-d_6) 1.14 (t, J = 7.3, 3H), 2.64 (q, J = 7.4, 2H), 7.31 (s, 1H), 7.11 (s, 1H), 11.91 (s, 1H), 11.91 (s, 1H); HRMS calcd for $C_{10}H_9ClN_2O_2$ 224.0348, found 224.0359. Anal. Calcd ($C_{10}H_9ClN_2O_2$) C, H, N.

6,7-Diethyl-1,4-dihydroquinoxaline-2,3-dione (5c): mp >360 °C; ^{1}H NMR (DMSO-d_6) 1.09 (t, J = 7.5 Hz, 6H), 2.51 (q, J = 7.5 Hz, 4H), 6.87 (s, 2H), 11.74 (s, 2H). Anal. ($C_{12}H_{14}N_2O_2$) C, H, N.

1,4-Dihydro-6,7-(methylenedioxy)quinoxaline-2,3-dione (5e): mp >360 °C; ^{1}H NMR (DMSO-d_6) 6.00 (s, 2H), 6.78 (s, 2H), 11.70 (s, 2H); HRMS calcd for $C_9H_6N_2O_4$ 206.2304, found 206.2306. Anal. ($C_9H_6N_2O_4$) C, H, N.

1,4-Dihydrobenzo[*g*]quinoxaline-2,3-dione (5f): mp >250 °C; ^{1}H NMR (DMSO-d_6) 7.38 (dd, J = 3.2, 6.2, 2H), 7.53 (s, 2H), 7.82 (dd, J = 3.2, 6.2, 2H), 12.09 (s, 2H). Anal. ($C_{12}H_8N_2O_2 \cdot H_2O$) C, H, N.

1,4-Dihydrocyclopento[*g*]quinoxaline-2,3-dione (5g): mp >360 °C; ^{1}H NMR (DMSO-d_6) 1.99 (m, 2H), 2.81 (t, J = 7.3, 4H), 6.957 (s, 2H), 11.83 (s, 2H); HRMS calcd for $C_{11}H_{10}N_2O_2$ 202.0738, found 202.0747. Anal. ($C_{11}H_{10}N_2O_2$) C, H, N.

1,4-Dihydro-7-methyl-5-nitroquinoxaline-2,3-dione (5h): mp 319−323 °C dec; ^{1}H NMR (DMSO-d_6) 2.35 (s, 3H), 7.27(s, 1H), 7.75 (s, 1H), 11.04 (s, 1H), 12.30 (s, 1H). Anal. ($C_9H_7N_3O_4 \cdot 0.35H_2O$) C, H, N.

1,4-Dihydro-6,7-dihydroxyquinoxaline-2,3-dione (5j). To a suspension of **5d** (222 mg, 1.0 mmol) in 2 mL of methylene chloride was added 5 mL of a solution of boron tribromide in methylene chloride (1 M, Aldrich). The resulting mixture was allowed to stir at room temperature for 24 h. The mixture was poured into ice−water (10 g) to form a suspension. Aqueous sodium hydroxide (20%, 10 mL) was added to the suspension to form a red solution. Then the solution was acidified with 6 N HCl (10 mL) to pH = 1. The suspension was centrifuged, washed with methanol, and dried *in vacuo* to give 170 mg (88%) of **5j** as a brown solid: mp >350 °C; ^{1}H NMR (DMSO-d_6) 6.54 (s, 2H), 8.99 (s, 2H), 11.54 (s, 2H); HRMS calcd for $C_8H_6N_2O_4$ 194.1480, found 194.1475. Anal. ($C_8H_6N_2O_4$) C, H, N.

6-Chloro-1,4-dihydro-7-methylquinoxaline-2,3-dione (5k). A suspension of 7-chloro-3,4-dihydro-6-methylquinoxalin-2(1*H*)-one[18] (38 mg, 0.19 mmol), 30% H_2O_2 (0.48 mL), and AcOH (0.8 mL) was stirred at room temperature for 3 days. Cream-colored solid was collected by vacuum filtration, washed with water (5 mL), and dried *in vicuo* to give 18 mg (44%) of **5k** as cream-colored powder: mp > 365 °C; ^{1}H NMR (DMSO-d_6) 2.27 (s, 3H), 7.02 (s, 1H), 7.11 (s, 1H), 11.89 (s, 1H), 11.95 (s, 1H); Anal. ($C_9H_7ClN_2O_2 \cdot 0.72H_2O$) C, H: calcd, 3.81; found, 3.24; N.

1,4-Dihydro-6,7-dimethyl-5-nitroquinoxaline-2,3-dione (9a). To a suspension of 1.90 g (10.0 mmol) of **5a** in 50 mL of TFA was added 1.104 g (11.0 mmol) of KNO_3 at room temperature, and the mixture was stirred at room temperature for 24 h. The resulting solution was evaporated to dryness, and the residue was treated with 30 mL of MeOH and evaporated again to dryness. The solid was treated with 20 mL of water, filtered, washed with water, and dried to give 1.86 g (79%) of **9a** as a yellow solid: mp 326−328 °C; ^{1}H NMR (DMSO-d_6) 2.08 (s, 3H), 2.25 (s, 3H), 7.05 (s, 1H), 11.77 (s, 1H), 12.03 (s, 1H). Anal. ($C_{10}H_9N_3O_4$) C, H, N.

QXs **9c,g,b (**mixture of **14b** and **14c)** were obtained from nitration of the corresponding 6,7-disubstituted QX in a manner similar to QX **9a**.

6,7-Diethyl-1,4-dihydro-5-nitroquinoxaline-2,3-dione (9c): mp 270−272 °C dec; ^{1}H NMR (DMSO-d_6) 1.14 (m, 6H), 2.62 (m, 4H), 7.09 (s, 1H), 11.76 (s, 1H), 12.03 (s, 1H). Anal. ($C_{12}H_{13}N_3O_4$) C, H, N.

1,4-Dihydro-5-nitrocyclopento[*g*]quinoxaline-2,3-dione (9g): mp 310 °C dec; ^{1}H NMR (DMSO-d_6) 2.04 (m, 2H), 2.97 (t, J = 7.5, 2H), 3.09 (t, J = 7.5, 2H), 7.26 (s, 1H), 11.18

(s, 1H), 12.23 (s, 1H); HRMS calcd for $C_{11}H_9N_3O_4$ 247.0588, found 247.0593. Anal. ($C_{10}H_6N_4O_4 \cdot 0.5H_2O$) C, H; N: calcd, 16.44; found, 15.77.

6-Chloro-7-ethyl-1,4-dihydro-5-nitroquinoxaline-2,3-dione (14b) and 7-Chloro-6-ethyl-1,4-dihydro-5-nitroquinoxaline-2,3-dione (14c): ^{1}H NMR (DMSO-d_6) 1.14 (m, 3H), 2.58 (q, J = 7.4, 1H), 2.71 (q, J = 7.4, 1H), 7.19 (s, 0.5H), 7.30 (s, 0.5H), 12.00 (mb, 0.5H), 12.17 (mb, 1.5H).

Ethyl *N*-(3-Bromo-4-ethyl-6-nitrophenyl)glycinate (12a). A mixture of 452 mg (1.81 mmol) of **8a**, 396 mg (2.87 mmol) of K_2CO_3, and 2.0 mL (3.0 g, 18 mmol) of ethyl bromoacetate was heated at 137 °C for 3 days. The mixture was cooled to room temperature, 12 mL of aqueous 1 N NaOH was added dropwise, and the mixture was stirred at room temperature for 1 h. The mixture was filtered, washed with water, and dried to leave 620 mg (100%) of a black solid: ^{1}H NMR ($CDCl_3$) 1.22 (t, J = 7.6, 3H), 1.33 (t, J = 7.2, 3H), 2.68 (q, J = 7.4, 2H), 4.05 (d, J = 5.4, 2H), 4.29 (m, 2H), 6.92 (s, 1H), 8.05 (s, 1H), 8.24 (sb, 1H). It was used for the next reaction without purification.

6-Bromo-7-ethyl-3,4-dihydroquinoxalin-2(1*H*)-one (13a). A solution of 620 mg (1.84 mmol) of **12a**, 1.62 g (7.18 mmol) of $SnCl_2 \cdot 2H_2O$, and 6 mL of absolute alcohol was refluxed for 4 h, and the mixture was cooled to room temperature. It was evaporated, and the residue was treated with aqueous 1 N NaOH to pH = 10. The mixture was filtered, washed with water, and dried to leave a solid. The solid was stirred with 20 mL of ethyl acetate for 1 h and filtered. The filtrate was evaporated to leave 342 mg (72%) of a yellow solid: ^{1}H NMR ($CDCl_3$) 1.17 (t, J = 7.4, 3H), 2.63 (q, J = 7.4, 2H), 3.80 (s, 1H), 3.95 (s, 2H), 6.55 (s, 1H), 6.86 (s, 1H), 7.83 (s, 1H). It was used for the next reaction without purification.

6-Bromo-7-ethyl-1,4-dihydro-5-nitroquinoxaline-2,3-dione (14a). To a solution of 321 mg (1.24 mmol) of **13a** in 6 mL of CF_3CO_2H stirred in an ice bath was added dropwise 1.5 mL of fuming HNO_3, and the solution was stirred in an ice bath for 1 h and at room temperature overnight. It was added into 30 mL of ice−water, and the precipitate was filtered, washed with water, and dried to leave 211 mg of a yellow solid. The solid was stirred with 20 mL of 1 N NaOH for 1 h and filtered. The filtrate was acidified by 6 N HCl to pH = 1. The precipitate was filtered, washed with water, and dried to leave 174 mg (44%) of yellow solid: mp >290 °C; ^{1}H NMR (DMSO-d_6) 1.16 (t, J = 7.4, 3H), 2.72 (q, J = 7.4, 2H), 7.19 (s, 1H), 12.20 (sb, 2H); HRMS calcd for $C_{10}H_8BrN_3O_4$ 312.9695, found 312.9711. Anal. ($C_{10}H_8BrN_3O_4 \cdot 0.1H_2O$) C, H, N.

6-Chloro-7-ethyl-1,4-dihydro-5-nitroquinoxaline-2,3-dione (14b). QX **14b** was prepared from **8b** in a manner similar to that of **14a**: mp >330 °C; ^{1}H NMR (DMSO-d_6) 1.16 (t, J = 7.4, 3H), 2.71 (q, J = 7.6, 2H), 7.19 (s, 1H), 12.21 (mb, 2H); HRMS calcd for $C_{10}H_8ClN_3O_4$ 269.0198, found 269.0196. Anal. ($C_{10}H_8ClN_3O_4$) C, H, N.

2,5-Dichloroethylbenzene (10c). A suspension of mossy zinc (10 g) and mercuric chloride (0.5 g) in concentrated HCl (0.5 mL) and water (5 mL) was shaken for ∼5 min. The aqueous layer was then decanted. To the residue was added concentrated HCl (7.5 mL) and water (7.5 mL), followed by 2′,5′-dichloroacetophenone (3.106 g, 16.43 mmol) and the suspension was refluxed for 4 h during which time, hourly addition of concentrated HCl (90.5 mL) was carried out. The resulting suspension was cooled to room temperature, and the aqueous layer was decanted. The residual solid was washed with ether (3 × 30 mL). The aqueous layer was extracted with ether (3 × 50 mL). The combined ether layer was washed with brine, dried over anhydrous Na_2SO_4, and removed *in vacuo*. The residue was dried further *in vacuo* to give 2.664 g of crude product which was purified on silica gel (hexane) to give 0.813 g (28%) of **10c** as a colorless liquid: ^{1}H NMR (DMSO-d_6) 1.23 (t, J = 7.5, 3H), 2.73 (q, J = 7.5, 2H), 7.09−7.27 (m, 3H).

2,5-Dichloro-4-ethylnitrobenzene (11c). To a stirred solution of **10c** (0.795 g, 4.68 mmol) in concentrated H_2SO_4 (4.5 mL) at 0 °C was added KNO_3 (0.473 g, 4.68 mmol) in one portion. The resulting pale yellow solution was allowed to warm to room temperature and was stirred overnight at room temperature. It was then poured into ice (80 g) and extracted with ether (3 × 30 mL). The extract was dried over anhydrous Na_2SO_4 and evaporated *in vacuo*, and the resulting oil was dried further *in vacuo* to give 0.943 g (92%) of **11c** as an oil: ^{1}H NMR ($CDCl_3$) 1.27 (t, J = 7.5, 3H), 2.80 (q, J = 7.5, 2H), 7.42 (s, 1H), 7.94 (s, 1H).

***N*-(4-Chloro-5-ethyl-2-nitrophenyl)glycine (12c).** To a solution of **11c** (0.585 g, 2.66 mmol) in ethanol (10.0 mL) at room temperature was added a solution of sodium glycinate (0.260 g, 2.68 mmol) in water (2.5 mL), and the resulting suspension was refluxed for 2 days. The solution was then cooled to room temperature, and the precipitated red solid was collected by filtration, washed with ethanol (4 mL), and dried *in vacuo* to give 0.086 g (13%) of **12c** as a red powder: ^{1}H NMR (DMSO-d_6) 1.15 (t, J = 7.2, 3H), 2.63 (q, J = 7.2, 2H), 3.47 (d, J = 3.0, 2H), 6.77 (s, 1H), 7.97 (s, 1H), 8.77 (s, 1H). The unreacted 2,5-dichloro-4-ethylnitrobenzene was recovered quantitatively from the filtrate.

7-Chloro-6-ethyl-3,4-dihydroquinoxalin-2(1*H*)-one (13c). A suspension of **12c** (0.082 g, 0.31 mmol) and $SnCl_2 \cdot 2H_2O$ (0.215 g, 0.953 mmol) in ethanol (1.0 mL) was refluxed for 45 min. It was then cooled to room temperature, and the precipitated solid was collected by filtration, washed with ethanol (1.0 mL), and dried *in vacuo* to yield 0.048 g (72%) of **13c** as a light yellow powder: ^{1}H NMR (DMSO-d_6) 1.05 (t, J = 7.2, 3H), 2.50−2.57 (m, 2H), 3.67 (s, 2H), 6.04 (s, 1H), 6.54 (s, 1H), 6.67 (s, 1H), 10.25 (s, 1H).

7-Chloro-6-ethyl-1,4-dihydro-5-nitroquinoxaline-2,3-dione (14c). QX **14c** was prepared from **13c** in a manner similar to that for **14a**: mp 302−306 °C dec; ^{1}H NMR (DMSO-d_6) 1.09 (t, J = 7.2, 3H), 2.52 (q, J = 7.2, 2H), 7.27 (s, 1H), 11.99 (s, 1H), 12.15 (s, 1H); HRMS calcd for $C_{10}H_8ClN_3O_4$ 269.0203, found 269.0190. Anal. ($C_{10}H_8ClN_3O_4$) C, H; N: calcd, 15.58; found, 14.40.

***N*-(4-Cyano-5-methyl-2-nitrophenyl)glycine (12d).** Glycine **12d** was prepared from 4-chloro-2-methylbenzonitrile (**10d**) in two steps similar to the preparation of **12c**: ^{1}H NMR (DMSO-d_6) 2.37 (s, 3H), 3.51 (d, J = 3.9, 1H), 6.85 (s, 1H), 8.38 (s, 1H), 9.13 (s, 1H).

7-Cyano-3,4-dihydro-6-methylquinoxalin-2(1*H*)-one (13d). To a stirred solution of **12d** (0.100 g, 0.389 mmol) in water (5.0 mL) at 100 °C was added sodium dithionite (0.540 g, 3.10 mmol) in two equal portions over 10 min. The resulting white suspension was stirred at 100 °C for 1 h and then cooled to room temperature. The solid was collected by filtration *in vacuo*, washed with water (4 mL), and dried *in vacuo* to give 0.040 g (55%) of **13d** as an off white powder: ^{1}H NMR (DMSO-d_6) 2.22 (s, 3H), 3.83 (s, 2H), 6.51 (s, 1H), 6.81 (br s, 1H), 6.83 (s, 1H), 10.38 (s, 1H).

7-Cyano-1,4-dihydro-6-methyl-5-nitroquinoxaline-2,3-dione (14d). A solution of **13d** (0.030 g, 0.16 mmol), fuming HNO_3 (0.070 mL), and TFA (0.700 mL) was stirred overnight at room temperature. The resulting solution was poured into ice water (7 mL), and the solid was collected by filtration *in vacuo*, washed with water (7 mL), and dried *in vacuo* to give 0.027 g of a 2:1 mixture of 7-cyano-1,4-dihydro-6-methyl-7-nitroquinoxaline-2,3-dione and 7-cyano-6-methyl-5-nitroquinoxalin-2(1*H*)-one as a yellow powder: ^{1}H NMR (DMSO-d_6) 2.34 (s, 3H), 2.41 (s, 3H), 7.47 (s, 1H), 7.75 (s, 1H), 8.35 (s, 1H), 12.21 (br s, 1H), 12.28 (s, 1H), 12.93 (s, 1H). This mixture was again stirred in fuming HNO_3 (0.10 mL) and TFA (1.0 mL) at room temperature for 72 h. The solution was poured into ice water (10 mL), and the solid was collected by filtration *in vacuo*, washed with water (10 mL), and dried *in vacuo* to give 0.018 g (36%) of **14d** as yellow powder: mp 341 °C dec; ^{1}H NMR (DMSO-d_6) 2.39 (s, 3H), 7.47 (s, 1H), 12.21 (br s, 1H), 12.27 (s, 1H); HRMS calcd for $C_9H_6N_4O_4$ 246.0388, found 246.0375.

***N*-(4-Cyano-2-nitrophenyl)glycine (12i).** Glycine **12i** was prepared from 4-chloro-3-nitrobenzonitrile in a manner similar to that of **12c**: ^{1}H NMR (DMSO-d_6) 3.510 (d, J = 3.9, 2H), 6.92 (d, J = 9.0, 1H), 7.72 (dd, J = 1.2, 9.0, 1H), 8.45 (d, J = 1.5, 1H), 9.19 (s, 1H).

7-Cyano-3,4-dihydroquinoxalin-2(1*H*)-one (13i). QX-2-one **13i** was prepared from **12i** in a manner similar to that for **13d**: ^{1}H NMR (DMSO-d_6) 3.86 (s, 2H), 0.641 (d, J = 8.4, 1H), 6.90 (s, 2H), 7.12 (dd, J = 1.2, 8.4, 1H), 10.47 (s, 1H).

7-Cyano-1,4-dihydro-5-nitroquinoxaline-2,3-dione (14i). QX **14i** was prepared from **13i** in a manner similar to that for

14a: mp 339−40 °C dec; ^{1}H NMR (DMSO-d_6) 7.64 (d, J = 1.2, 1H), 8.34 (d, J = 1.5, 1H), 11.47 (s, 1H), 12.47 (s, 1H). Anal. ($C_9H_6N_4O_4 \cdot 0.3H_2O$) C, H, N.

5-Amino-7-chloro-1,4-dihydro-6-methylquinoxaline-2,3-dione (15). A solution of QX **14g**[18] (75 mg, 0.29 mmol) and $SnCl_2 \cdot 2H_2O$ (200 mg, 0.88 mmol) in ethanol (1.0 mL) was refluxed for 6.5 h. The reaction mixture was cooled to room temperature and allowed to stand overnight at room temperature. The yellow solid was collected by filtration *in vacuo*, washed with ethanol (1.5 mL), and dried *in vacuo* to give 54 mg (81%) of **15** as a yellow powder: mp 323 °C dec; ^{1}H NMR (DMSO-d_6) 2.09 (s, 3H), 5.47 (s, 2H), 6.46 (s, 1H), 11.15 (s, 1H), 11.72 (s, 1H). Anal. ($C_9H_6N_4O_4 \cdot 0.88\ H_2O$) C, H; N: calcd, 17.40; found, 16.32.

6-Amino-7-fluoro-1,4-dihydro-5-nitroquinoxaline-2,3-dione (18a). To a solution of 20 mg (0.082 mmol) of **16**[10] in 0.5 mL of DMSO-d_6 was added 2 drops of 30% ammonium hydroxide, and the solution was heated at 80 °C for 24 h. To the mixture was added one more drop of 30% ammonium hydroxide, and it was heated at 80 °C for 10 h. The mixture was added into 4 mL of water and acidified with 2 N HCl to pH = 1. The precipitate was filtered, washed with water, and dried to leave 15 mg (76%) of a yellow solid: mp 250 °C dec; ^{1}H NMR (DMSO-d_6) 7.20 (d, J = 11.5, 1H), 7.23 (sb, 2H), 11.15 (mb, 1H), 12.0 (mb, 1H); ^{19}F NMR −133.84 (d, J = 11.6); HRMS calcd for $C_8H_5FN_4O_4$ 240.0290, found 240.0294. Anal. ($C_8H_5FN_4O_4 \cdot 0.6H_2O$) C, H; N: calcd, 22.32; found, 21.00.

7-Fluoro-1,4-dihydro-6-methoxy-5-nitroquinoxaline-2,3-dione (18b). To a mixture of 162 mg (0.667 mmol) of **16** and 162 mg (2.85 mmol) of sodium methoxide was added 4 mL of DMSO, and the solution was stirred at room temperature overnight. The solution was diluted with 15 mL of water and acidified with 2 N HCl to pH = 4. The precipitate was filtered, washed with water, and dried to leave 157 mg (92%) of a yellow solid: mp >300 °C; ^{1}H NMR (DMSO-d_6) 3.90 (s, 3H), 7.16 (d, J = 11.6, 1H), 11.97 (mb, 1H), 12.14 (s, 1H); ^{19}F NMR −134.48 (mb). Anal. ($C_9H_6FN_3O_5$) C, H, N.

6-Ethoxy-7-fluoro-1,4-dihydro-5-nitroquinoxaline-2,3-dione (18c). QX **18c** was prepared from **16** and sodium ethoxide in a manner similar to the preparation of **18b**: mp 293−5 °C; ^{1}H NMR (DMSO-d_6) 1.24 (t, J = 7.0, 3H), 4.14 (q, J = 7.2, 2H), 7.15 (d, J = 11.7, 1H), 11.98 (s, 1H), 12.14 (s, 1); ^{19}F NMR −134.06 (mb); HRMS calcd for $C_{10}H_8FN_3O_5$ 269.0443, found 269.0454. Anal. ($C_{10}H_8FN_3O_5 \cdot 0.2H_2O$) C, H, N.

6-*n*-Butoxy-7-fluoro-1,4-dihydro-5-nitroquinoxaline-2,3-dione (18d). To a mixture of 25 mg (1.0 mmol) of NaH and 50 mg (0.67 mmol) of 1-butanol was added 1.5 mL of DMSO, and the mixture was stirred for 1 h. To the resulting solution was added 27 mg (0.11 mmol) of **16**, and the solution was stirred at room temperature for 5 days. The solution was diluted with 3 mL of water and acidified with 2 N HCl to pH = 1. The precipitate was filtered, washed with water, and dried to leave 11 mg (33%) of a yellow solid: mp 290−292 °C; ^{1}H NMR (DMSO-d_6) 0.88 (t, J = 7.4, 3H), 1.36 (m, 2H), 1.60 (m, 2H), 4,08 (t, J = 6.3, 2H), 7.15 (d, J = 11.54, 1H), 11.98 (m, 1H), 12.12 (m, 1H); HRMS calcd for $C_{12}H_{12}FN_3O_5$ 297.0755, found 297.0757. Anal. ($C_{12}H_{12}FN_3O_5 \cdot 0.2H_2O$) C, H, N.

7-Fluoro-1,4-dihydro-5-nitro-6-(3-phenylpropoxy)quinoxaline-2,3-dione (18e). QX **18e** was prepared in a manner similar to that for **18d**: mp 280−2 °C; ^{1}H NMR (DMSO-d_6) 1.92 (m, 2H), 2.65 (t, J = 7.8, 2H), 4.10 (t, J = 6.1, 2H), 7.14−7.31 (m, 6H), 11.98 (m, 1H), 12.131 (s, 1H); ^{19}F NMR −133.87 (mb); HRMS calcd for $C_{17}H_{14}FN_3O_5$ 359.0911, found 359.0927. Anal. ($C_{17}H_{14}FN_3O_5 \cdot 0.2H_2O$) C, H, N.

7-Chloro-1,4-dihydro-6-methoxy-5-nitroquinoxaline-2,3-dione (18f). QX **18f** was prepared from **17**[10] in a manner similar to that for **18b**: mp > 316 °C; ^{1}H NMR (DMSO-d_6) 3.82 (s, 3H), 7.28 (s, 1H), 12.08 (s, 1H), 12.12 (s, 1H). Anal. ($C_9H_6ClN_3O_5 \cdot 0.1$DMSO) C, H, N.

7-Chloro-1,4-dihydro-5-nitro-6-thioethoxyquinoxaline-2,3-dione (18g). A solution of **17** (0.100 g, 0.385 mmol) and ethanethiol sodium salt (0.130 g, 1.55 mmol) in DMSO (1.0 mL) was stirred at room temperature for 24 h. The resulting solution was diluted with water (5 mL) and acidified with concentrated HCl (4 or 5 drops) to pH ∼5. The precipitated solid was collected by filtration *in vacuo*, washed with water (10 mL), and dried *in vacuo* to obtain 0.098 g (84%) of **18g** as a yellow powder: mp >323 °C; ^{1}H NMR (DMSO-d_6) 1.04 (t, J = 7.2, 3H), 2.82 (q, J = 7.5, 2H), 7.34 (s, 1H), 12.20 (s, 1H), 12.25 (s, 1H); HRMS calcd for $C_{10}H_8ClN_3O_4S$ 300.9924, found 300.9919.

Pharmacology

DCKA Binding Assay. The potency of the QXs as inhibitors of [^{3}H]DCKA binding was determined in rat brain cortical membranes as previously described.[19] 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 in the presence of nine concentrations of test compound added in 5 μL DMSO (1% final). Nonspecific binding was defined using ACEA 1021 (10 μM). The assays were terminated by rapid filtration, and filter-bound radioactivity was determined by liquid scintillation spectrometry. IC_{50} values were determined using the sigmoidal equation in Prism (GraphPad).

Electrophysiology. 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.[8] 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). Previously unreported 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.[27]

Mouse Maximum Electroshock-Induced Seizure Tests. Procedures for the mouse MES assay were as reported previously.[11,14] QXs **2**, **9a**, **14a,b,e−h**, and **21** were dissolved in 0.05 M Tris and were tested for anticonvulsant effects at the peak of activity which occurred 2 min after iv administration. For QX **14a** the onset of activity was slower and the compound was tested at a peak of activity occurring 15 min after injection. ED_{50} values were determined by Litchfield and Wilcoxon analysis.

log *P* Measurement. log P values for QXs **2**, **9a**, and **14a,e,g** were obtained by classic shake-flask method in octanol vs pH 7.4 buffer. Eight QXs with log P values between −0.97 and 1.04 obtained from classic shake-flask method were chromatographed at three concentrations, and the log K'_0 for each compound was determined. A linear plot of log K'_0 vs log P was made from these eight QXs and used for the estimation of log P values from log K'_0 values. log P values for QXs **14f,h** and **21** were obtained by the HPLC method and estimated from their log K'_0 values. The log P value for QX **14b** was calculated on the basis of the log P of **14a** (1.04) and the log P difference between **14f** and **14g** (0.12).

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