Synthesis and Structure−Activity Relationships of 1,2,3,4-Tetrahydroquinoline-2,3,4-trione 3-Oximes: Novel and Highly Potent Antagonists for NMDA Receptor Glycine Site

Sui Xiong Cai,*,† Zhang-Lin Zhou,‡ Jin-Cheng Huang,† Edward R. Whittemore,† Zizi O. Egbuwoku,† Yixin Lü,‡ Jon E. Hawkinson,† Richard M. Woodward,† Eckard Weber,† and John F. W. Keana‡

CoCensys Inc., 213 Technology Drive, Irvine, California 92618, and Department of Chemistry, University of Oregon, Eugene, Oregon 97403

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A series of 1,2,3,4-tetrahydroquinoline-2,3,4-trione 3-oximes (QTOs) was synthesized and evaluated for antagonism of NMDA receptor glycine site. Glycine site affinity was determined using a [^{3}H]DCKA binding assay in rat brain membranes and electrophysiologically in *Xenopus* oocytes expressing 1a/2C subunits of cloned rat NMDA receptors. Selected compounds were also assayed for antagonism of AMPA receptors in *Xenopus* oocytes expressing rat brain poly-(A)$^+$ RNA. QTOs were prepared by nitrosation of 2,4-quinolinediols. Structure−activity studies indicated that substitutions in the 5-, 6-, and 7-positions increase potency, whereas substitution in the 8-position causes a decrease in potency. Among the derivatives evaluated, 5,6,7-trichloro-QTO was the most potent antagonist with an IC_{50} of 7 nM in the [^{3}H]DCKA binding assay and a K_b of 1−2 nM for NMDA receptors expressed in *Xenopus* oocytes. 5,6,7-Trichloro-QTO also had a K_b of 180 nM for AMPA receptors in electrophysiological assays. The SAR of QTOs was compared with the SAR of 1,4-dihydroquinoxaline-2,3-diones (QXs). For compounds with the same benzene ring substitution pattern, QTOs were generally 5−10 times more potent than the corresponding QXs. QTOs represent a new class of inhibitors of the NMDA receptor which, when appropriately substituted, are among the most potent glycine site antagonists known.

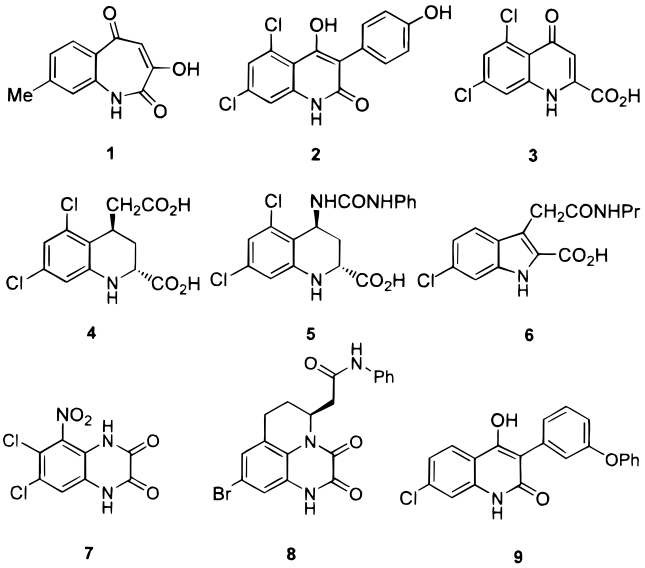
Introduction

The *N*-methyl-D-aspartate (NMDA) receptor is a ligand-gated ion channel which requires simultaneous binding of both glutamate and glycine for activation.[1] NMDA receptor-mediated excitotoxicity is implicated in a number of pathophysiological conditions which lead to neuronal death,[2] including acute ischemic brain damage[3] and neurodegenerative conditions such as Alzheimer's disease and AIDS-related dementia.[4] A variety of approaches for therapeutic intervention have been developed based on inhibition of NMDA receptor activation using (a) competitive glutamate site antagonists, (b) noncompetitive ion channel blockers, (c) antagonists for the glycine coagonist site, and (d) ligands for the inhibitory polyamine sites and other noncompetitive allosteric binding sites.[5]

The discovery that NMDA receptor glycine site antagonists may lack certain undesirable side effects observed with channel blockers, such as MK-801, has stimulated research in the development of glycine site antagonists as a means of reducing glutamate-induced excitotoxicity.[6] A number of potent antagonists for the glycine site have been reported[7] including benzazepine **1**,[8] 4-hydroxy-3-phenylquinolin-2(1*H*)-one **2**,[9] kynurenic acid **3**,[10] *trans*-2-carboxy-4-(carboxymethyl)tetrahydroquinoline **4**,[11] 2-carboxy-4-(phenylureido)tetrahydroquinoline **5**,[12] 2-carboxy-3-indoleacetamide **6**,[13] quinoxaline-2,3-dione (QX) **7**,[14] tricyclic quinoxaline-2,3-dione **8**,[15] and 3-(3-phenoxyphenyl)-4-hydroxyquinolin-2(1*H*)-one **9**[16] (Chart 1).

We have previously reported a series of substituted QXs, leading to ACEA 1021 (**7**), and related trisubstituted QXs, which are potent glycine site antagonists.[17] From our structure−activity relationship (SAR) studies of QXs, we noted that one of the two amide NHs is essential for activity. To explore other heterocycles as novel antagonists of the glycine site, we elected to retain the NH at the 1-position and the C=O at the 2-position of the QX and to replace the C=O at the 3-position and the NH at the 4-position with other functional groups. Herein we report that 1,2,3,4-tetrahydroquinoline-2,3,4-trione 3-oximes (QTOs) **11** are a novel class of potent glycine site antagonists.

Chart 1



Chemistry

The starting 2,4-quinolinediols were prepared as shown in Scheme 1. For instance, 5,7-dichloro-2,4-

† CoCensys Inc.
‡ University of Oregon.
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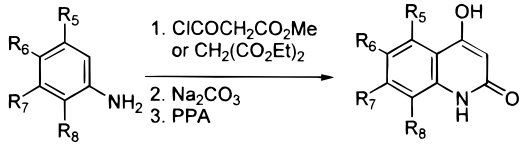
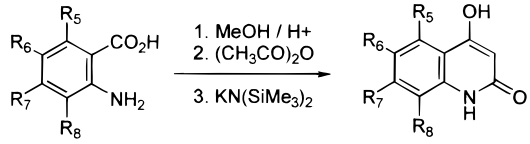
Scheme 1



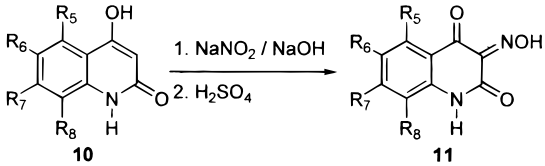
10f, $R_5 = R_6 = Cl$, $R_7 = R_8 = H$
10g, $R_5 = R_7 = Cl$, $R_6 = R_8 = H$
10h, $R_6 = R_7 = Cl$, $R_5 = R_8 = H$
10i, $R_5 = R_6 = R_7 = Cl$, $R_8 = H$
10j, $R_5 = R_7 = Me$, $R_6 = R_8 = H$
10k, $R_5 = H$, $R_6 = R_8 = F$, $R_7 = Cl$
10i, $R_5 = R_6 = R_7 = R_8 = F$

Scheme 2



10b, $R_5 = R_6 = R_8 = H$, $R_7 = Cl$
10c, $R_5 = R_7 = R_8 = H$, $R_6 = Cl$
10d, $R_6 = R_7 = R_8 = H$, $R_5 = Cl$
10e, $R_5 = R_6 = R_7 = H$, $R_8 = Cl$

Scheme 3



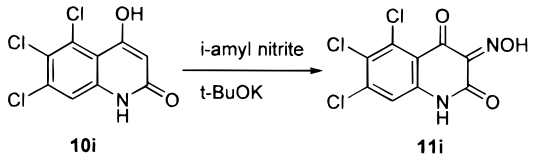
quinolinediol (**10g**) was prepared by heating 3,5-dichloroaniline with diethyl malonate followed by basic hydrolysis and cyclization in polyphosphoric acid (PPA), according to the procedure of Patel and Mehta.[18] For anilines multisubstituted with electron-withdrawing groups, it was better to react the aniline with ethyl malonyl chloride. For instance, 5,6,7,8-tetrafluoro-2,4-quinolinediol (**10l**) was prepared by reaction of 2,3,4,5-tetrafluoroaniline with ethyl malonyl chloride followed by basic hydrolysis and cyclization in PPA.

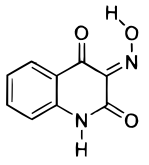
When an aniline was unsymmetrically substituted, a mixture of two products was obtained after cyclization. Thus, reacting 3,4-dichloroaniline with diethyl malonate, followed by hydrolysis and cyclization, gave a 1:1 mixture of 5,6-dichloro-2,4-quinolinediol (**10f**) and 6,7-dichloro-2,4-quinolinediol (**10h**).[19]

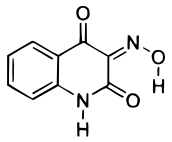
Alternatively, certain 2,4-quinolinediols were prepared from substituted 2-aminobenzoic acids. For instance, 2-amino-4-chlorobenzoic acid was converted to the ester and then to the acetamide which was cyclized by treatment with $KN(SiMe_3)_2$ (Scheme 2)[20] to give 7-chloro-2,4-quinolinediol (**10b**).

QTOs were synthesized by nitrosation of the respective 2,4-quinolinediols. For instance, QTO (**11a**) was prepared by reaction of 2,4-quinolinediol (**10a**) with $NaNO_2/H_2SO_4$ (Scheme 3), according to the procedure of Dahn and Donzel.[21] ^1H NMR showed that **11a** was a mixture of syn−anti isomers, owing to the two stereoisomers about the C=N double bond in the oxime. All the other QTOs prepared were also a mixture of syn−anti isomers, and the ratio of syn−anti isomers was close to 1:1 in most cases as determined by ^1H NMR. Under the normal ^1H NMR scanning range (down to 14 ppm), only one D_2O exchangeable proton (two signals around 10−11 ppm due to syn−anti isomers) was observed. This proton most probably is the amide NH because the amide NH signals in QXs (cf. NMR spectrum of QX **12j**) are also around 10−11 ppm. However, when the ^1H NMR scanning range was extended to 16 ppm, one more D_2O exchangeable proton (two signals due to syn−anti isomers) was observed around 15−16 ppm (e.g., QTO **11c**), indicating that one of the D_2O exchangeable protons in QTO, presumably to be the oxime NOH, is more acidic than the amide NH.

Scheme 4




anti **11a**


syn **11a**

Nitrosation of the mixture of **10f**,**h** gave a mixture of **11f**,**h**, which was separated by fractional crystallization in ethanol. The ^1H NMR spectrum of the 5,6-dichloro isomer **11f** showed that the two aromatic protons are coupled to each other, while the ^1H NMR spectrum of the 6,7-dichloro isomer **11h** showed no coupling between the two aromatic protons.

Reaction of 5,6,7-trichloro-2,4-quinolinediol (**10i**) with $NaNO_2/H_2SO_4$ failed to produce the oxime, possibly due to low solubility of **10i** in water. Alternatively, nitrosation of **10i** was carried out with isoamyl nitrite in *t*-BuOK[22] to give 5,6,7-trichloro-QTO (**11i**) (Scheme 4).

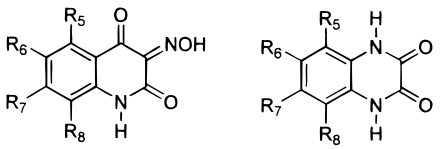
Pharmacology

The affinity of QTOs for the NMDA receptor glycine site was measured by inhibition of [^3H]-5,7-dichlorokynurenic acid ([^3H]DCKA) binding to rat brain cortical membranes.[23] For selected QTOs, potencies at NMDA receptor glycine sites and at α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors were determined electrophysiologically in *Xenopus* oocytes expressing 1a/2C subunits of NMDA receptors and rat brain poly(A)$^+$ RNA,[17] respectively. Apparent antagonist dissociation constants (K_b values) were estimated by assuming competitive inhibition at the glycine site 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. In the case of compound **11i**, the apparent K_b value at NMDA glycine sites was also calculated from the rightward shift of glycine concentration−response curves induced by fixed concentrations of antagonist.

Results and Discussion

The unsubstituted QTO (**11a**) was the first compound prepared in the series. Its low micromolar affinity for NMDA receptor glycine sites was comparable to that of the unsubstituted QX (**12a**). SAR studies in the QX series have shown that appropriate multiple substitu-

Table 1. SAR of Substituted 1,2,3,4-Tetrahydroquinoline-2,3,4-trione 3-Oximes **11** and 1,4-Dihydroquinoxaline-2,3-diones **12** at NMDA Receptor Glycine Sites



					[^{3}H]DCKA IC_{50} (μM)[a]	
no.	R_5	R_6	R_7	R_8	**11**	**12**
a	H	H	H	H	1.4 ± 0.2	9.8 ± 0.6
b	H	H	Cl	H	0.053 ± 0.003	1.8 ± 0.3
c	H	Cl	H	H	3.4 ± 0.3	*b*
d	Cl	H	H	H	4.4 ± 0.4	ND[c]
e	H	H	H	Cl	>100	ND
f	Cl	Cl	H	H	0.17 ± 0.03	ND
g	Cl	H	Cl	H	0.033 ± 0.009	0.28 ± 0.02
h	H	Cl	Cl	H	0.012 ± 0.001	0.13 ± 0.03
i	Cl	Cl	Cl	H	0.007 ± 0.001	0.030 ± 0.005
j	Me	H	Me	H	0.037 ± 0.004	2.1 ± 0.1
k	H	F	Cl	F	0.91 ± 0.08	0.63 ± 0.11
l	F	F	F	F	3.3 ± 0.7	0.73 ± 0.05

[a] Affinity is expressed as IC_{50} estimates for inhibition of [^{3}H]DCKA binding to rat brain membranes. Values are means ± SEMs of at least three independent experiments. [b] The structure of **12c** is the same as **12b**. [c] ND, not determined.

tion in the benzene portion of the molecule can enhance the affinity by as much as several hundred fold.[17] We therefore explored the SAR of QTOs by systematic substitution in the benzene portion of the molecule (Table 1).

Potency among the monochloro-substituted QTOs varied more than 1000-fold depending on the position of the chlorine atom. The most potent one was 7-chloro-substituted QTO **11b**, demonstrating the importance of substitution in the 7-position for high-affinity inhibition. This observation is similar to SARs in several other series of glycine site antagonists. For example, the most potent kynurenic acids include a chlorine atom in the 7-position.[10] The 5- and 6-chloro-substituted QTOs **11d**,**c** were about as active as the unsubstituted QTO (**11a**), whereas 8-chloro-QTO (**11e**) was essentially inactive. We interpret this as an indication that an unencumbered NH group in the QTOs is critical for tight binding to the receptor, probably via a hydrogen-bonding interaction. As seen with QXs,[17] substitution in the 8-position probably interferes with this interaction.

Three dichloro-substituted QTOs were found to exhibit high-affinity binding for sites labeled by [^{3}H]-DCKA. QTOs **11g**,**h**, each possessing a chlorine atom in the 7-position, were considerably more potent than 5,6-dichloro-QTO (**11f**) and somewhat more potent than 7-chloro-QTO (**11b**). 5,6,7-Trichloro-QTO (**11i**) was the most potent compound in this series with an IC_{50} of 7 nM in the [^{3}H]DCKA binding assay. In comparison, the precursor to **11i**, namely, 5,6,7-trichloro-2,4-quinolinediol (**10i**) was more than 1000-fold less potent (IC_{50} = 9 ± 1 μM). Interestingly, 5,7-dimethyl-QTO (**11j**) was about as potent as 5,7-dichloro-QTO (**11g**), showing the comparability of Me with Cl and implying that it is the size and/or hydrophobicity of the substituents, and not their electronic properties, that are the main determinants of potency for QTOs. This is a deviation from most of the known glycine site antagonists in which an

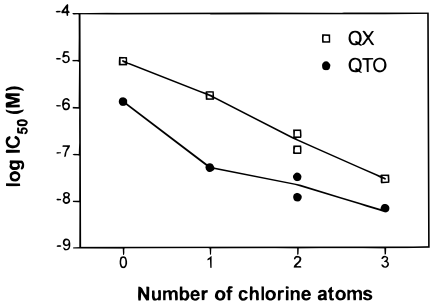


Figure 1. Effect of chlorine substitution in QXs and QTOs. The log of the IC_{50} value in the [^{3}H]DCKA binding assay (Table 1) is plotted for unsubstituted, monosubstituted (R7), disubstituted (R_5, R_7 and R_6, R_7), and trisubstituted (R_5, R_6, R_7) QTOs and QXs.

electron-withdrawing group such as Cl in the 7-position is preferred.[24]

In contrast, compounds with multiple substitutions which included the 8-position had low affinity. Thus 7-chloro-6,8-difluoro-QTO (**11k**) is less potent than 7-chloro-QTO (**11b**), and 5,6,7,8-tetrafluoro-QTO (**11l**) is even less potent than the unsubstituted parent compound **11a**. These results further support the notion that the NH is critical for binding to the receptor.

The SAR of QTOs was compared with the SAR of QXs[17] (Table 1). For compounds with the same substituents, QTOs generally had higher affinity than QXs. Figure 1 shows the effect of chlorine atom substitution on potency for both QTOs and QXs. Note the progressive increase in potency as chlorine atoms are substituted in the 7-position and, additionally, in the 5- and 6-positions. In this series, the QTOs were consistently about 10 times more potent than the corresponding QXs.

It is interesting to note that 5,7-dimethyl-QTO (**11j**) is about 50 times more potent than 5,7-dimethyl-QX (**12j**). This is a reflection of the lower potency of 5,7-dimethyl-QX (**12j**) vs 5,7-dichloro-QX (**12g**), since 5,7-dimethyl-QTO (**11j**) and 5,7-dichloro-QTO (**11g**) have similar potencies. We interpret this as an indication that the presence of an electron-withdrawing group may not be as critical for the potency of QTOs as it is for the potency of QXs.

Interestingly, tetrafluoro-QTO (**11l**) is less active than tetrafluoro-QX (**12l**). Also, **11l** is less active than the unsubstituted QTO (**11a**), while **12l** is more potent than the unsubstituted QX (**12a**). These data further support the argument that electron-withdrawing groups are not critical for the potency of QTOs. The fluorine atoms in **11l** only serve to reduce the potency of QTO, while the fluorine atoms in **12l** are important as electron-withdrawing groups and increase potency in the QX series.

We also note that the 6,7,8-trisubstituted QTO **11k** and the corresponding 6,7,8-trisubstituted QX **12k** have similar potencies. This is mainly due to the low potency of the 6,7,8-trisubstituted QTO vs the other QTOs and indicates the importance of an accessible NH in binding to the receptor. On the other hand, the QXs possess two NH groups, either of which can interact with the receptor. In this respect, a 6,7,8-trisubstituted QX is equivalent to a 5,6,7-trisubstituted QX and contains an unhindered NH group for hydrogen bonding with the binding site.

The similarity between the SAR of QTOs and QXs suggests that QTOs and QXs probably interact with the

Table 2. Functional Antagonism by QTOs **11** of NR1a/2C NMDA Receptors and Rat Brain AMPA Receptors Expressed in *Xenopus* Oocytes

	11				**12**[e]	
no.	glycine K_b (μM)[a]	AMPA K_b (μM)[b]	selectivity for NMDA[c]	n[d]	glycine K_b (μM)	AMPA K_b (μM)
b	0.086[f] (0.085−0.087)[g]	0.74 (0.59−0.93)	8.6	3, 3	2.6	16
g	0.047 (0.041−0.054)	0.60 (0.50−0.73)	13	4, 4	0.73	9.3
h	0.024 (0.022−0.026)	0.41 (0.29−0.57)	17	4, 3	0.38	4.8
i	0.0022 (0.0017−0.0029)	0.18 (0.16−0.21)	82	3, 4	0.082	4.2
j	0.17 (0.15−0.19)	2.6 (2.2−3.0)	15	3, 3	ND[h]	ND

[a] Inhibition of NMDA receptors was measured in oocytes expressing the rat subunit combination NR1a/2C. 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 sites were estimated from inhibition of currents elicited by 10 μM AMPA. [c] The steady-state selectivity index for inhibition at NMDA receptor glycine sites was estimated by dividing K_b(AMPA) by K_b(NMDA). [d] Indicates the number of independent experiments (cells examined); numbers refer to NMDA and AMPA, respectively. [e] Data from ref 17 for QXs. [f] K_b values are given to two significant figures. [g] Numbers in parentheses are 95% confidence intervals adjusted to the linear scale. [h] ND, not determined.

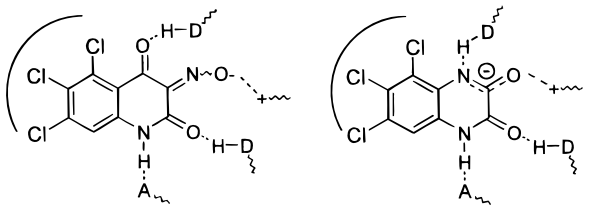


Figure 2. Proposed hydrogen-bonding and charge−charge interactions of QTOs and QXs with the glycine site of NMDA receptor. A represents a receptor hydrogen bond acceptor, and D−H represents a receptor hydrogen bond donor; + represents a positively charged receptor group.

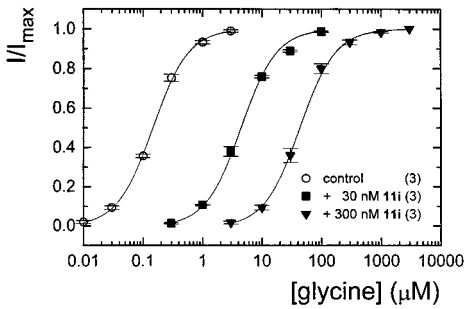


Figure 3. Inhibition of NR1a/2C receptors by **11i** surmountable by glycine. Glycine concentration−response curves were generated with glutamate fixed at 100 μM. Curves were first measured under control conditions and then repeated in two concentrations of **11i**. EC_{50} and slope values for the three curves measured independently were control, 0.15 μM and 1.5; 30 nM **11i**, 4.5 μM and 1.3; 300 nM **11i**, 43 μM and 1.6. Smooth curves are the simultaneous fit optimized for the three sets of data; slope value 1.5. Amplitudes of maximum responses ranged between 77 and 264 nA (mean = 173 + 24 nA; n = 9).

NMDA receptor glycine site in a similar manner. On the basis of the above SAR, we propose the binding model for QTOs and QXs[10] shown in Figure 2. The model contains the following features: (1) an NH group in the 1-position as a hydrogen bond donor, (2) the C=O in the 2-position as a hydrogen bond acceptor, (3) a charge−charge interaction at the 3-position, (4) a hydrogen bond acceptor in the 4-position, and (5) hydrophobic interactions in the benzene ring portion of the molecule. Explanations for why QTOs generally have higher potency than QXs could include (a) better charge−charge interaction in the 3-position and/or (b) better hydrogen-bonding interaction in the 4-position. Since the amide NH in QX is not very acidic,[25] substitution of electron-withdrawing groups in the benzene ring can increase the acidity of the NH and thus increase the potency of QXs. On the other hand, QTOs are reasonably acidic,[25] presumably due to the oxime NOH; therefore the electronic properties of the substituents in the benzene ring have little effect on the potency of QTOs.

Functional antagonism of NMDA receptors by five selected QTOs was tested in *Xenopus* oocytes expressing the cloned rat NMDA receptor subunit combination NR1a/2C using electrophysiological recording techniques.[26] Apparent dissociation constants (K_b values) for QTOs were estimated from partial concentration−inhibition curves (Table 2) (see the Experimental Section). For **11b**,**g**,**h**, potency of inhibition was less than 2-fold different from that measured in the [^{3}H]DCKA binding assay and followed the same rank order. For **11i** potency in the electrophysiological assay was ~3-fold higher than that in [^{3}H]DCKA binding and for **11j** ~5-fold lower. Response amplitudes for these experiments ranged between 475 and 755 nA (mean = 627 ± 25 nA; n = 17).

In order to unequivocally demonstrate that **11i** is a glycine site antagonist, a second series of experiments was done wherein the K_b value was determined from shifts in glycine concentration−response curves induced by fixed concentrations of antagonist (Figure 3). Analysis of these data by simultaneous fit gave a K_b value for **11i** of 1.0 (0.9−1.1) nM (n = 3), which is close to the K_b (2.2 nM) value obtained from partial concentration−inhibition curve. Though the data did not strictly conform to the simple competitive model ($F_{2,27}$ = 4.37),[26] the set of curves is essentially parallel, indicating that inhibition is predominant at the glycine site. These data confirm that **11i** is a high-potency inhibitor of NR1a/2C receptors. The modest discrepancy with the [^{3}H]-DCKA binding assays is probably because NR1a/2C receptors, a predominantly cerebellar subtype,[27] have higher affinity for **11i** than the major subtypes of NMDA receptor in rat forebrain. Indeed, this trend is apparent with ACEA 1021, a high-potency QX, which is about 3-fold more potent at NR1a/2C than at the mixture of receptors expressed in oocytes by rat whole brain poly(A)$^+$ RNA.[26] The lower potency measured for **11j** is less readily explained but may also be due to comparing inhibition at a single receptor subtype with net potency for a heterogeneous population of receptors. As seen in the binding studies, comparison of K_b values for the four compounds tested suggests that, for a given pattern of halogen substitutions on the benzene ring, QTOs are generally more potent than QXs. Using previously estimated K_b values (Table 2),[17] **11g**,**h** are 16-fold, **11b** 30-fold, and **11i** 37-fold more potent than the corresponding QXs. These comparisons are not direct,

however, because the QX values were calculated on NMDA responses expressed by whole brain poly(A)$^+$ RNA.

Electrophysiological recordings were also used to test the five selected QTOs for functional antagonism of AMPA responses in oocytes expressing rat cerebral cortex poly(A)$^+$ RNA. K_b values for QTOs were again estimated from partial concentration−inhibition curves (Table 2). Amplitudes of control responses ranged between 32 and 194 nA (mean = 113 ± 14 nA; n = 17). Potency of inhibition for AMPA receptors followed the same rank order as that for NMDA receptor glycine sites. The range of potency at AMPA receptors was, however, narrower than at NMDA: ∼14-fold for AMPA as compared to ∼80-fold at glycine sites. 5,6,7-Trichloro-QTO (**11i**) is the most potent antagonist with a K_b of 180 nM. As was the case with NMDA receptors, QTOs are generally more potent than the corresponding QXs as antagonists of AMPA receptors; **11g** is 16-fold, **11h** 12-fold, and **11b**,**i** ∼22-fold more potent than the corresponding QXs (Table 2). Interestingly, the increase in potency at AMPA sites roughly parallels that seen at NMDA glycine sites. Consequently, levels of selectivity for QTOs are comparable to the corresponding levels for QXs.[17] As seen with QXs, the highest potency glycine site antagonist (**11i**) also shows the highest level of selectivity for NMDA versus AMPA. These experiments indicate that QTOs, particularly those with moderate potency, should not be considered as highly selective ligands for NMDA receptors. Generalizing from the selected compounds, most QTOs have moderate (10−20-fold) selectivity for NMDA glycine sites over AMPA receptors. However, QTOs with appropriate substitution resulted in high potency (e.g., **11i**) and ∼80-fold selectivity for the glycine site.

Conclusion

In conclusion, a series of 1,2,3,4-tetrahydroquinoline-2,3,4-trione 3-oximes (QTOs) was synthesized and shown to be a novel class of potent antagonists for NMDA receptors acting at the glycine recognition site. Substitution in the 5,6,7-positions of QTOs in general substantially increases affinity for the glycine site. Substitution in the 8-position by chlorine sharply reduces potency, indicating the importance of the NH for interacting with the receptor. 5,6,7-Trichloro-QTO (**11i**) has an IC_{50} value of 7 nM in the [^{3}H]DCKA binding assay and a K_b of 1−2 nM for 1a/2C NMDA receptors, indicating that it is one of the most potent antagonists of the NMDA receptor glycine site reported to date. Although selective for the glycine site, QTO **11i** also has a K_b of 180 nM at the AMPA receptor, a potency approaching the most potent AMPA antagonists reported.[24]

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. Elemental analyses were performed by Desert Analytics, Tucson, AZ. Mass spectra (MS) were obtained with a VG 12-250 or VG ZAB-2FHF mass spectrometer. Substituted anilines, aminobenzoic acids, and 2,4-quinolinediol were obtained from Aldrich 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. For the intermediate 2,4-quinolinediols **10** that are characterized by HRMS, HPLC purities are >95%. Compounds **12a**,**b**,**f**−**i**,**k** have been reported previously.[17]

5,7-Dichloro-2,4-quinolinediol (10g). A solution of 11.6 g (71.6 mmol) of 3,5-dichloroaniline and 26.3 g (164 mmol) of diethyl malonate was heated at 180 °C for 6 h, and the ethanol produced was collected in a Dean−Stark trap (∼3.5 mL). The solution was cooled to room temperature, and a precipitate was observed. The mixture was filtered, and the solid was washed with methanol (40 mL). The combined filtrate and wash was mixed with 26 g (245 mmol) of Na_2CO_3 and 200 mL of water and refluxed for 1 h. The solution was cooled in an ice bath and then acidified to pH = 1 with aqueous 2 N HCl. The mixture was filtered, washed with water, and dried to leave a white solid (13.9 g). The solid was mixed with 150 mL of polyphosphoric acid and heated at 140 °C for 3 h. The solution was cooled to room temperature and diluted with 200 mL of aqueous 1 N HCl. The mixture was stirred for 4 h and then neutralized with aqueous 20% NaOH to pH = 4. It was filtered, and the solid was washed with water and dried to leave 12.3 g (74%) of **10g** as a white solid: mp 360−361 °C dec; ^{1}H NMR ($CDCl_3$ + DMSO-d_6) 5.45 (s, 1H), 6.62 (d, J = 1.8, 1H), 6.80 (d, J = 1.8, 1H), 10.92 (bs, 1H); HRMS calcd for $C_9H_5{}^{35}Cl_2NO_2$ 228.9694, found 228.9709.

Compounds **10f**,**h**,**k**,**j** were similarly prepared from the corresponding substituted anilines.

5,6-Dichloro-2,4-quinolinediol (10f) and 6,7-dichloro-2,4-quinolinediol (10h): mp 350 °C dec (lit.[19] mp 335 °C); ^{1}H NMR ($CDCl_3$ + DMSO-d_6) for **10f** 5.60 (s, 1H), 6.85 (d, J = 9.0, 1H), 7.07 (d, J = 9.0, 1H), 10.90 (bs, 1H), for **10h** 5.51 (s, 1H), 7.05 (s, 1H), 7.51 (s, 1H), 10.86 (sb, 1H); **10f:10h** = 1:1.

7-Chloro-6,8-difluoro-2,4-quinolinediol (10k): mp > 250 °C; ^{1}H NMR (DMSO-d_6) 5.83 (s, 1H), 7.54 (d, J = 9.34, 1H), 11.49 (bm, 1H), 11.84 (bm, 1H); HRMS calcd for $C_9H_4{}^{35}ClF_2NO_2$ 230.9896, found 230.9890.

5,7-Dimethyl-2,4-quinolinediol (10j): mp 353−356 °C dec (lit.[28] mp > 365 °C); ^{1}H NMR (DMSO-d_6) 2.25 (s, 3H), 2.62 (s, 3H), 5.61 (s, 1H), 6.70 (d, 1H), 6.88 (d, 1H), 11.02 (s, 1H); HRMS calcd for $C_{11}H_{11}NO_2$ 189.0790, found 189.0783.

5,6,7,8-Tetrafluoro-2,4-quinolinediol (10l). To a solution of 1.02 g (6.19 mmol) of 2,3,4,5-tetrafluoroaniline and 0.706 g (6.99 mmol) of triethylamine in 10 mL of dry CH_2Cl_2 kept in an ice bath was added dropwise a solution of 0.997 g (6.62 mmol) of ethyl malonyl chloride (Aldrich) in 2 mL of CH_2Cl_2. The mixture was stirred at room temperature overnight. It was washed with water (4 × 20 mL), dried, and evaporated to leave 1.59 g (92%) of an almost colorless solid: ^{1}H NMR ($CDCl_3$) 1.34 (t, 3H), 3.52 (s, 2H), 4.30 (q, 2H), 8.12 (m, 1H), 9.81 (s, 1H).

The solid was mixed with 0.82 g of Na_2CO_3, 10 mL of water, and 6 mL of methanol and refluxed for 1.5 h. The solution was cooled to room temperature and acidified by aqueous 2 N HCl in an ice bath to pH = 2−3. The mixture was filtered, washed with water, and dried to leave 820 mg (57%) of a white solid: ^{1}H NMR ($CDCl_3$) 3.60 (s, 2H), 8.10 (m, 1H), 9.42 (s, 1H).

A mixture of 410 mg of the solid with 15 mL of PPA was heated at 140 °C for 6 h. The solution was cooled to room temperature, diluted with 10 mL of aqueous 1 N HCl and 10 mL of water, and stirred at room temperature for 10 h. The solution was neutralized with aqueous 20% NaOH to pH = 3. It was filtered, washed with water, dried to leave 96 mg (25%) of **10l** as a white solid: mp > 360 °C (lit.[29] mp > 360 °C); ^{1}H NMR (DMSO-d_6) 5.79 (s, 1H), 11.65 (bs, 1H), 11.78 (s, 1H); HRMS calcd for $C_9H_3F_4NO_2$ 233.0098, found 233.0102.

5,6,7-Trichloro-2,4-quinolinediol (**10i**). Compound **10i** was similarly prepared from 3,4,5-trichloroaniline: mp 303−305 °C dec; ^{1}H NMR (DMSO-d_6) 2.45 (s, 1H), 5.78 (s, 1H), 7.42 (s, 1H), 11.51 (s, 1H), 11.66 (s, 1H). Anal. ($C_9H_4Cl_3NO_2$) C, H, N.

7-Chloro-2,4-quinolinediol (10b). A solution of 3.457 g (20 mmol) of 2-amino-4-chlorobenzoic acid in methanol (50 mL) and concentrated sulfuric acid (2 mL) was refluxed for 12 h.

The solution was evaporated to give a solid which was treated with 100 mL of water. The mixture was filtered, washed with water (3 × 15 mL), 10% $NaHCO_3$ (15 mL), and water (3 × 15 mL), and dried in vacuo to give 3.5 g (94%) of methyl 2-amino-4-chlorobenzoate as a white solid: mp 66−68 °C; ^{1}H NMR ($CDCl_3$) 3.86 (s, 1H), 5.56 (bs, 2H), 6.56 (d, J = 8.7, 1H), 6.67 (s, 1H), 7.76 (d, J = 8.4, 1H).

A solution of 2.095 g (11.3 mmol) of methyl 2-amino-4-chlorobenzoate and acetic anhydride (12.5 mL) in dry dioxane (20 mL) was heated (50 °C) under nitrogen for 3 h. Water (5 mL) was then added, and the solution was evaporated to leave a white solid which was collected by filtration and dried to give 1.976 g (77%) of methyl 2-acetamido-4-chlorobenzoate: mp 121−123 °C; ^{1}H NMR ($CDCl_3$) 2.24 (s, 3H), 3.93 (s, 3H), 7.03 (dd, J = 8.7, 1.8, 1H), 7.94 (d, J = 8.7, 1H), 8.81 (d, J = 1.8, 1H), 11.10 (s, 1H).

A solution of 113.8 mg (0.5 mmol) of methyl 2-acetamido-4-chlorobenzoate in dry THF (2 mL) was added dropwise into a solution of potassium bis(trimethylsilyl)amide in toluene (3 mL, 1.5 mmol) at −78 °C under nitrogen. The reaction mixture was then allowed to warm to room temperature and stirred at room temperature for 12 h. The mixture was poured into ice/water (5 mL). The aqueous layer was washed with ethyl acetate (5 mL) and acidified (pH = 2) to give a white solid which was collected by filtration and dried in vacuo, giving 80 mg (82%) of **10b**: mp 278−280 °C dec (lit.[30] mp 279 °C); ^{1}H NMR (DMSO-d_6) 5.69 (s, 1H), 7.11 (dd, J = 8.6, 1.8, 1H), 7.24 (d, J = 1.8, 1H), 7.71 (d, J = 8.6, 1H), 11.25 (s, 1H), 11.46 (s, 1H); HRMS calcd for $C_9H_6{}^{35}ClNO_2$ 195.0084, found 195.0079.

Compounds **10c**−**e** were similarly prepared from the corresponding chloro-substituted 2-aminobenzoic acids.

6-Chloro-2,4-quinolinediol (10c): mp 342−344 °C dec (lit.[28] mp > 360 °C); ^{1}H NMR (DMSO-d_6) 5.72 (s, 1H), 7.22 (d, J = 8.7, 1H), 7.48 (dd, J = 8.7, 2.4, 1H), 7.68 (d, J = 2.4, 1H), 11.30 (s, 1H), 11.50 (s, 1H); HRMS calcd for $C_9H_6{}^{35}ClNO_2$ 195.0084, found 195.0088.

5-Chloro-2,4-quinolinediol (10d): mp 346−348 °C dec; ^{1}H NMR (DMSO-d_6) 5.74 (s, 1H), 7.11 (d, J = 7.5, 1H), 7.18 (d, J = 8.4, 1H), 7.34 (dd, J = 8.4, 7.5, 1H), 11.34 (s, 1H), 11.37 (s, 1H); HRMS calcd for $C_9H_6{}^{35}ClNO_2$ 195.0084, found 195.0088.

8-Chloro-2,4-quinolinediol (10e): mp 280−282 °C dec (lit.[28] mp 305 °C); ^{1}H NMR (DMSO-d_6) 5.76 (s, 1H), 7.10 (dd, J = 8.1, 7.2, 1H), 7.61 (d, J = 7.2, 1H), 7.76 (d, J = 8.1, 1H), 10.36 (s, 1H), 11.60 (s, 1H); HRMS calcd for $C_9H_6{}^{35}ClNO_2$ 195.0084, found 195.0083.

5,7-Dichloro-1,2,3,4-tetrahydroquinoline-2,3,4-trione 3-Oxime (11g). To a mixture of 147 mg (0.616 mmol) of 5,7-dichloro-2,4-quinolinediol (**10g**) and 126 mg (1.82 mmol) of sodium nitrite in 3 mL of 0.2 N NaOH in an ice bath was added dropwise 2 mL of aqueous 2 N H_2SO_4. The mixture was stirred in the ice bath for 4 h after addition of H_2SO_4, filtered, and dried to leave an orange solid. The solid was crystallized by boiling with 10 mL of ethanol (95%). The solution was cooled to room temperature, filtered, and dried to leave 132 mg (83%) of **11g** as a yellow solid: mp 244−245 °C dec; ^{1}H NMR ($CDCl_3$ + DMSO-d_6) 7.01 (d, J = 1.8, 0.44H), 7.08 (m, 1.54H), 11.57 (bs, 0.45H), 11.82 (s, 0.55H); HRMS calcd for $C_9H_4{}^{35}Cl_2N_2O_3$ 257.9595, found 257.9598. Anal. ($C_9H_4Cl_2N_2O_3$) C, H; N: calcd, 10.81; found, 10.36.

Compounds **11a**−**e,j**−**l** were similarly prepared from the corresponding 2,4-quinolinediols **10**.

1,2,3,4-Tetrahydroquinoline-2,3,4-trione 3-oxime (11a): mp 206 °C dec (lit.[21] mp 208 °C dec); ^{1}H NMR ($CDCl_3$ + DMSO-d_6) 6.65−6.78 (m, 2H), 7.15 (m, 1H), 7.56 (d, J = 8.1, 0.3H), 7.61 (d, J = 8.1, 0.7H), 10.89 (s, 0.3H), 11.35 (s, 0.7H); HRMS calcd for $C_9H_6N_2O_3$ 190.0375, found 190.0400. Anal. ($C_9H_6N_2O_3$) C, H, N.

7-Chloro-1,2,3,4-tetrahydroquinoline-2,3,4-trione 3-oxime (11b): mp 230−232 °C dec; ^{1}H NMR (DMSO-d_6) 7.10 (m, 2H), 7.81 (m, 1H), 11.18 (s, 0.5H), 11.40 (s, 0.5H); HRMS calcd for $C_9H_5{}^{35}ClN_2O_3$ 223.9985, found 223.9993. Anal. ($C_9H_5ClN_2O_3 \cdot 0.45H_2O$) C, H, N.

6-Chloro-1,2,3,4-tetrahydroquinoline-2,3,4-trione 3-oxime (11c): mp 245−247 °C dec (lit.[31] mp 163 °C); ^{1}H NMR (DMSO-d_6) 7.15 (m, 1H), 7.68−7.81 (m, 2H), 11.22 (s, 0.5H), 11.52 (s, 0.5H), 14.97 (s, 0.5H), 15.64 (s, 0.5H); ^{1}H NMR (DMSO-d_6 + D_2O) 7.12 (m, 1H), 7.64−7.78 (m, 2H); HRMS calcd for $C_9H_5{}^{35}ClN_2O_3$ 223.9985, found 223.9991. Anal. ($C_9H_5ClN_2O_3 \cdot 0.2H_2O$) C, H; N: calcd, 12.30; found, 11.86.

5-Chloro-1,2,3,4-tetrahydroquinoline-2,3,4-trione 3-oxime (11d): mp 220−222 °C dec; ^{1}H NMR (DMSO-d_6) 7.04−7.18 (m, 2H), 7.49 (m, 1H), 11.20 (s, 0.5H), 11.43 (s, 0.5 H); HRMS calcd for $C_9H_5{}^{35}ClN_2O_3$ 223.9985, found 223.9984. Anal. ($C_9H_5ClN_2O_3$) C, H, N.

8-Chloro-1,2,3,4-tetrahydroquinoline-2,3,4-trione 3-oxime (11e): mp 180−182 °C; ^{1}H NMR (DMSO-d_6) 7.11 (m, 1H), 7.72 (m, 2H), 10.31 (s, 0.5H), 10.51 (s, 0.5H); HRMS calcd for $C_9H_5{}^{35}ClN_2O_3$ 223.9985, found 223.9986. Anal. ($C_9H_5ClN_2O_3 \cdot 0.1H_2O$) C, H, N.

5,7-Dimethyl-1,2,3,4-tetrahydroquinoline-2,3,4-trione 3-oxime (11j): mp 228−230 °C; ^{1}H NMR (DMSO-d_6) 2.27 (s, 1.4H), 2.28 (s, 1.6H), 2.54 (s, 1.4H), 2.57 (s, 1.6H), 6.75−6.82 (m, 2H), 11.07 (s, 0.54H), 11.37 (s, 0.46H); HRMS calcd for $C_{11}H_{10}N_2O_3$ 218.0690, found 218.0682. Anal. ($C_{11}H_{10}N_2O_3 \cdot 0.4H_2O$) C, H, N.

7-Chloro-6,8-difluoro-1,2,3,4-tetrahydroquinoline-2,3,4-trione 3-oxime (11k): mp 240 °C dec; ^{1}H NMR (DMSO-d_6) 7.68 (m, 1H), 11.34 (bm, 0.6H), 11.50 (bm, 0.4H); HRMS calcd for $C_9H_3{}^{35}ClF_2N_2O_3$ 259.9797, found 259.9785. Anal. ($C_9H_3ClF_2N_2O_3$) C, H; N: calcd, 10.75; found, 10.20.

5,6,7,8-Tetrafluoro-1,2,3,4-tetrahydroquinoline-2,3,4-trione 3-oxime (11l): mp 220−221 °C dec; ^{1}H NMR (DMSO-d_6) 11.48 (s, 0.7H), 11.54 (m, 0.3H); HRMS calcd for $C_9H_2F_4N_2O_3$ 261.9999, found 261.9981. Anal. ($C_9H_2F_4N_2O_3$) C, H, N.

5,6-Dichloro-1,2,3,4-tetrahydroquinoline-2,3,4-trione 3-Oxime (11f) and 6,7-Dichloro-1,2,3,4-tetrahydroquinoline-2,3,4-trione 3-Oxime (11h). A mixture of 113 mg (0.491 mmol) of **10f**,**h** and 120 mg (1.73 mmol) of $NaNO_2$ in 3 mL of 0.2 N NaOH was stirred for 30 min. To the resulting solution was added dropwise 1 mL of 2 N H_2SO_4. The resulting red mixture was stirred overnight, filtered and washed with water, and dried to leave 111 mg of a yellow solid. ^{1}H NMR showed **11f:11h** = 1:1. The solid was boiled with 15 mL of 95% ethanol and 2 mL of acetone, and the mixture was filtered. The solid in the filter was dried to leave 20 mg (15.7%) of **11h** as a yellow solid: mp > 250 °C; ^{1}H NMR ($CDCl_3$ + DMSO-d_6) 6.99 (s, 0.4H), 7.01 (s, 0.6H), 7.66 (s, 0.4H), 7.73 (s, 0.6H), 11.10 (s, 0.4H), 11.55 (s, 0.6H); HRMS calcd for $C_9H_4{}^{35}Cl_2N_2O_3$ 257.9595, found 257.9611. Anal. ($C_9H_4Cl_2N_2O_3$) H; C: calcd, 41.73; found, 42.46. N: calcd, 10.81; found, 10.21.

A solid precipitate was observed in the filtrate after it was cooled to room temperature. The precipitate was collected by filtration, washed with a minimum amount of ethanol, and dried to leave 37 mg of a yellow solid which was a mixture of **11f**,**h** as determined by ^{1}H NMR. A second crop of precipitate was observed in the filtrate. The precipitate was filtered and dried to leave 40 mg (31%) of **11f** as a yellow-orange solid: mp 218−219 °C; ^{1}H NMR (DMSO-d_6) 7.10 (d, J = 8.6, 0.5H), 7.12 (d, J = 8.8, 0.5H), 7.79 (d, J = 8.8, 0.5H), 7.83 (d, J = 8.8, 0.5H), 11.29 (s, 0.5H), 11.47 (s, 0.5H). Anal. ($C_9H_4Cl_2N_2O_3 \cdot 0.6H_2O$) C, H, N.

5,6,7-Trichloro-1,2,3,4-tetrahydroquinoline-2,3,4-trione 3-Oxime (11i). To a solution of 228 mg (0.86 mmol) of **10i** in *t*-BuOH (15 mL) were added *t*-BuOK (488 mg, 4.35 mmol) and isoamyl nitrite (0.585 mL, 4.35 mmol). The resulting mixture was stirred for 12 h at room temperature under N_2, and then cooled water (10 mL) was added slowly with stirring. To the resulting deep yellow solution was added 0.5 N HCl to adjust the pH to 2. The precipitate was filtered, washed with H_2O (6 × 10 mL), and dried at 40 °C under 0.1 mmHg for 10 h to give 220 mg (87%) of **11i** as a yellow powder: mp 270 °C dec; ^{1} H NMR (DMSO-d_6) 7.31 (s, 1H), 11.34 (s, 0.5H), 11.43 (s, 0.5H); HRMS calcd for $C_9H_3{}^{35}Cl_3N_2O_3$ 291.9210, found 291.9213. Anal. ($C_9H_3Cl_3N_2O_3$) C, H, N.

5,7-Dimethyl-1,4-dihydroquinoxaline-2,3-dione (12j). A solution of 1.66 g (10 mmol) of 4,6-dimethyl-2-nitroaniline in 35 mL of ethanol with 200 mg of 10% Pd/C was hydrogenated at 25 psi for 2 h. The catalyst was removed by filtration, and evaporation of the solvent gave 1.30 g (96%) of the diamine. A solution of 424 mg (3.12 mmol) of the diamine and 432 mg (3.43 mmol) of oxalic acid dihydrate in 20 mL of 4 N

HCl was refluxed for 3 h. The mixture was cooled to room temperature, filtered, and dried to give 516 mg (87%) of **12j**: mp 350−352 °C; ^{1}H NMR (DMSO-d_6) 2.21 (s, 3H), 2.28 (s, 3H), 6.75 (s, 1H), 6.77 (s, 1H), 11.17 (s, 1H), 11.89 (s, 1H); HRMS calcd for $C_{10}H_{10}N_2O_2$ 190.0741, found 190.0744. Anal. ($C_{10}H_{10}N_2O_2 \cdot 0.5H_2O$) C, H, N.

Pharmacology. DCKA Binding Assay. The [^{3}H]DCKA binding assay was performed according to the method of Canton et al.[23] with modifications. Rat brain cortices were obtained from ABS (Wilmington, DE) and stored at −80 °C. The thawed cortices were homogenized with a Polytron instrument (Brinkman) in 10 vol of 0.32 M sucrose and centrifuged at 1000*g* for 10 min at 4°C. All further steps were done at 4 °C except where noted. The resultant supernatant was centrifuged at 20000*g* for 20 min to obtain the P2 pellets. The P2 pellets were resuspended in water and centrifuged at 8000*g* for 20 min. The resultant supernatant and upper "buffy" layer were centrifuged at 50000*g* for 20 min. The pellets were washed once by resuspension in water and centrifugation at 50000*g* for 20 min and stored at −80 °C until use.

On the day of the assay, the thawed pellets were resuspended in 20 vol of assay buffer (50 mM HEPES−KOH, pH = 7.5), incubated for 20 min at 37 °C, and centrifuged at 50000*g* for 10 min. The pellets were washed four times by resuspension in 20 vol of assay buffer and centrifugation at 50000*g* for 10 min. The final pellets were resuspended in assay buffer to ∼2 mg/mL. Aliquots (200 μL) of the membrane suspension (400 μg of protein) were incubated with 15−20 nM [^{3}H]DCKA (15.8−16.9 Ci/mmol; New England Nuclear) and 5 μL aliquots of test compound (nine concentrations) dissolved in dimethyl sulfoxide (DMSO) (final DMSO concentration: 1%). The incubation was brought to a final volume of 0.5 mL with assay buffer. Nonspecific binding was determined in the presence of 1 mM ACEA 1021 and ranged from 20% to 35%. Following a 30 min incubation on ice, the assays were terminated by filtration through glass fiber filters (Schleicher and Schuell no. 32) using a cell harvester (Brandel) precooled with cold buffer and rinsed two times with ice-cold buffer. Filter-bound radioactivity was measured by liquid scintillation spectrometry. Protein was determined using a modified Lowry assay.[32] The IC_{50} (concentration of test compound producing 50% inhibition of specific binding) was estimated using the sigmoidal equation (Prism, GraphPad).

Electrophysiology. Oocytes were obtained from mature female *Xenopus laevis* and maintained as previously described.[17] Oocytes were microinjected with either a mixture of NR1a and NR2C cRNA (∼1 ng of each subunit/oocyte) or rat cerebral cortex poly(A)$^+$ RNA.[17] Clones were prepared and transcribed using conventional techniques.[26] Membrane current responses were recorded at −70 mV. All drugs were dissolved in Ringer solution and applied to the oocytes *via* a linear array of microcapillary tubes.[33] L-Glutamate and glycine were purchased from RBI. All other chemicals were purchased from Sigma. QTOs were initially dissolved in DMSO. Agonist concentration−response curves were analyzed as described previously.[26] For the values given in Table 2, the EC_{50} and slope values for glycine at NR1a/2C receptors were 0.17 μM and 1.45, respectively ($n = 6$) (curves not illustrated). Using AMPA as the agonist, the EC_{50} and slope values for AMPA receptors expressed by rat cerebral cortex poly(A)$^+$ RNA were 5.89 μM and 1.96, respectively ($n = 5$). The apparent dissociation constant (K_b value) for **11i** was determined from a simultaneous fit of concentration−response data in the absence of inhibitor and in 30 and 300 nM **11i**. Conformity to the simple competitive model was assessed using the *F*-test.[26] K_b values given in Table 2 were calculated from partial (3−5) point concentration−inhibition curves using a generalized form of the Cheng−Prussoff equation:[34]

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

where IC_{50} is the concentration of QTO 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 (glycine or AMPA) that elicits a half-maximal response, and n is the slope factor of the agonist concentration−response relation (see above). Fixed agonist concentrations were 1 μM glycine plus 100 μM glutamate for NMDA receptors and 10 μM AMPA for AMPA receptors. Other data quoted in the text and figure legends are given as mean ± SEM.

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