Structure−Activity Relationships of 4-Hydroxy-3-nitroquinolin-2(1*H*)-ones as Novel Antagonists at the Glycine Site of *N*-Methyl-D-aspartate Receptors

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Received July 18, 1996⊗

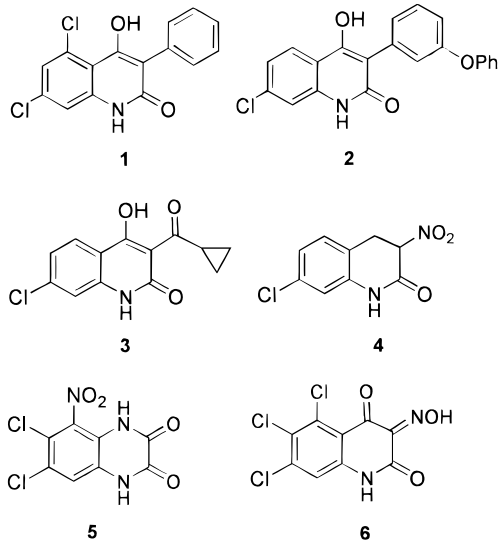
A series of 4-hydroxy-3-nitroquinolin-2(1*H*)-ones (HNQs) was synthesized by nitration of the corresponding 2,4-quinolinediols. The HNQs were evaluated as antagonists at the glycine site of NMDA receptors by inhibition of [^{3}H]DCKA binding to rat brain membranes. Selected HNQs were also tested for functional antagonism by electrophysiological assays in *Xenopus* oocytes expressing either 1a/2C subunits of NMDA receptors or rat brain AMPA receptors. The structure−activity relationships (SAR) of HNQs showed that substitutions in the 5-, 6-, and 7-positions in general increase potency while substitutions in the 8-position cause a sharp reduction in potency. Among the HNQs tested, 5,6,7-trichloro HNQ (**8i)** was the most potent antagonist with an IC_{50} of 220 nM in [^{3}H]DCKA binding assay and a K_b of 79 nM from electrophysiological assays. Measured under steady-state conditions HNQ **8i** is 240-fold selective for NMDA over AMPA receptors. The SAR of HNQs was compared with those of 1,4-dihydroquinoxaline-2,3-diones (QXs) and 1,2,3,4-tetrahydroquinoline-2,3,4-trione 3-oximes (QTOs). In general, HNQs have similar potencies to QXs with the same benzene ring substitution pattern but are about 10 times less active than the corresponding QTOs. HNQs are more selective for NMDA receptors than the corresponding QXs and QTOs. The similarity of the SAR of HNQs, QXs, and QTOs suggested that these three classes of antagonists might bind to the glycine site in a similar manner. With appropriate substitutions, HNQs represent a new class of potent and highly selective NMDA receptor glycine site antagonists.

Introduction

Excessive stimulation by excitatory amino acids such as glutamate is known to cause degeneration and death of neurons, a process termed excitotoxicity.[1] It is now generally accepted that *N*-methyl-D-aspartate (NMDA) receptors, a subclass of ionotropic glutamate receptors, play a major role in the excitotoxicity of glutamate.[2] The discovery that glycine is a necessary coagonist for NMDA receptor activation[3] has prompted a search for glycine antagonists[4] as potential central nervous system (CNS) therapeutic agents with anticonvulsant and neuroprotective properties.[5] Examples include 4-hydroxy-3-phenylquinolin-2(1*H*)-ones such as **1,**[6] 4-hydroxy-3-(3-phenoxyphenyl)quinolin-2(1*H*)-ones such as **2**,[7] and 3-acyl-4-hydroxyquinolin-2(1*H*)-ones such as **3**[8] which are selective glycine antagonists. In addition, 3,4-dihydro-3-nitroquinolin-2(1*H*)-ones such as **4**[9] (Chart 1)-are reported to have essentially equal potency as antagonists at NMDA receptor glycine sites and at α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, a subtype of the non-NMDA class of ionotropic glutamate receptors.

We have recently reported on the structure−activity relationship (SAR) of substituted 1,4-dihydroquinoxaline-2,3-diones (QXs) leading to ACEA 1021 (**5**), a highly potent and systemically active glycine site antagonist.[10] In search of other heterocyclic glycine site antagonists, we modified the QX heterocycle ring by preserving the NH and CO in the 1- and 2-positions while replacing the CO and NH in the 3- and 4-positions with other functional groups. These changes led to the discovery of 1,2,3,4-tetrahydroquinoline-2,3,4-trione 3-oximes (QTOs) such as **6,** which is a highly potent glycine site antagonist.[11] Herein we report that 4-hydroxy-3-nitroquinolin-2(1*H*)-ones (HNQs) **8** constitute a novel class of potent and selective NMDA receptor glycine site antagonists.

Chart 1



Chemistry

The HNQs **8** were synthesized by nitration (HNO_3/CH_3CO_2H) of the corresponding 2,4-quinolinediols **7**.[12] 6,7-Dichloro HNQ (**8g**) was obtained from nitration of a mixture of 6,7- and 5,6-dichloro-2,4-quinolinediols.[11]

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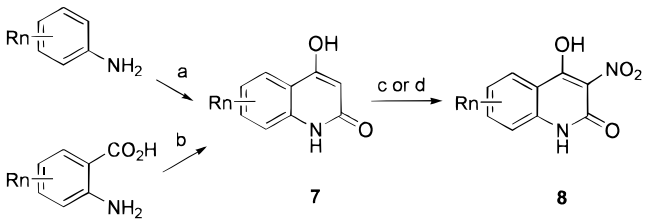
⊗ Abstract published in *Advance ACS Abstracts,* October 15, 1996.

S0022-2623(96)00520-1 CCC: $12.00

Scheme 1[a]



[a] (a) 1, EtO_2CCH_2COCl or $(EtO_2C)_2CH_2$, 2, NaOH, 3, PPA; (b) 1, MeOH, 2, $(CH_3CO)_2O$, 3, $KN(SiMe_3)_2$; (c) HNO_3/CH_3CO_2H; (d) KNO_3/CF_3CO_2H.

Nitration of 5,6,7- and 6,7,8-trichloro-2,4-quinolinediols (**7i**,**l**) was carried out in CF_3CO_2H instead of CH_3CO_2H owing to the poor solubility of the starting materials in CH_3CO_2H. 2,4-Quinolinediols **7b**−**l** were prepared from the corresponding anilines or 2-aminobenzoic acids as reported previously (Scheme 1).[11]

Pharmacology

The affinity of HNQs for the NMDA receptor glycine site was measured by inhibition of [^{3}H]-5,7-dichlorokynurenic acid ([^{3}H]DCKA) binding to rat brain cortical membranes.[11] In addition, apparent antagonist dissociation constants (K_b) of selected HNQs were determined by electrophysiological techniques using either cloned 1a/2C rat NMDA receptors expressed in *Xenopus* oocytes or AMPA receptors expressed in oocytes using whole rat brain poly(A)$^+$ RNA. K_b values were estimated by assuming competitive inhibition, as indicated by the binding assays, and measuring 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. The apparent K_b value of HNQ **8i** at the glycine site was also calculated from the rightward shift of the glycine concentration−response curves induced by fixed concentrations of antagonist.

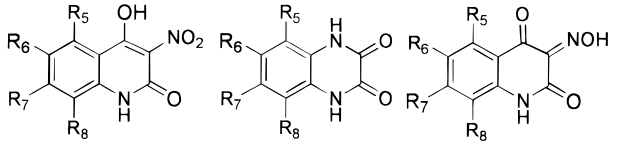
Results and Discussion

The SAR of HNQs as antagonists at the glycine site of NMDA receptors is shown in Table 1. Unsubstituted HNQ (**8a)** has low micromolar affinity for the glycine site, which is comparable to that of the unsubstituted QX (**9a**). Among the monochloro-substituted HNQs, 7-chloro HNQ (**8b)** is the most potent antagonist, consistent with the SAR of other classes of glycine antagonists.[11,13] The other monochloro-substituted HNQs either show very low potency (**8d)** or are devoid of activity (**8c**,**e**).

Two or more chlorine atoms in the 5-, 6-, and 7-positions enhanced the affinity of the HNQs 85−150-fold relative to the unsubstituted HNQ (**8a**). 5,6,7-Trichloro HNQ (**8i)** is among the most potent HNQs with an IC_{50} of 220 nM in the [^{3}H]DCKA binding assay. 6,7-Dichloro HNQ (**8g)** and 5,7-dichloro HNQ (**8f)** also have comparable potencies.

It is interesting to note that 5,7-dimethyl HNQ (**8h)** is about as potent as 5,7-dichloro HNQ (**8f),** indicating that the presence of an electron-withdrawing group is not critical for high potency in the HNQ series. This is different from most of the known glycine antagonists in which the presence of a polar or electron-withdrawing group in the 7-position is required for high potency.[14]

Table 1. Structure−Activity Relationship of HNQs **8** vs QXs **9** and QTOs **10**



8 **9** **10**

					[^{3}H]DCKA IC_{50} (μM)		
no.	R_5	R_6	R_7	R_8	**8**[a]	**9**[b]	**10**[b]
a	H	H	H	H	34 ± 3	9.8	1.4
b	H	H	Cl	H	1.5 ± 0.1	1.8	0.053
c	H	Cl	H	H	>100	d	3.4
d	Cl	H	H	H	61 ± 2	ND[c]	4.4
e	H	H	H	Cl	>100	ND	>100
f	Cl	H	Cl	H	0.22 ± 0.05	0.28	0.033
g	H	Cl	Cl	H	0.40 ± 0.02	0.13	0.012
h	Me	H	Me	H	0.29 ± 0.03	2.1	0.037
i	Cl	Cl	Cl	H	0.22 ± 0.04	0.030	0.007
j	H	F	Cl	F	40 ± 5	0.63	0.91
k	F	F	F	F	>100	0.73	3.3
l	H	Cl	Cl	Cl	>100	d	ND

[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] Data from ref 11. [c] ND, not determined. [d] Due to the symmetrical structure of QXs, **9c,i** are identical with **9b**,**l**, respectively.

Remarkably, 7-chloro-6,8-difluoro HNQ (**8j)** has very low affinity. 5,6,7,8-Tetrafluoro HNQ (**8k)** and 6,7,8-trichloro HNQ (**8l)** are inactive. The comparison of **8i** to **8l** is most striking. By moving the chlorine atom in the 5-position (**8i)** to the 8-position (**8l**), a 220 nM antagonist was converted into an inactive ligand, i.e., >400-fold reduction in potency. We interpret this as an indication that the NH in the 1-position of the HNQ is critical for binding of the ligand to the receptor, possibly by hydrogen-bonding, and substitution in the 8-position interferes with this critical interaction.

A comparison of the SAR of HNQs **8** with the SAR of QXs **9** shows that for the chloro-substituted compounds **b, f,** and **g**, HNQs and the corresponding QXs have similar affinity. 5,6,7-Trichloro HNQ (**8i)** is an exception, being about 7 times less active than the corresponding QX **9i.**

We note that 5,7-dimethyl HNQ (**8h)** is about 7-fold more potent than the corresponding 5,7-dimethyl QX (**9h)**. This is mainly due to the lower potency of 5,7-dimethyl QX (**9h)** vs 5,7-dichloro QX (**9f)**. Interestingly, 5,7-dimethyl QTO (**10h)** is also much more potent than the corresponding QX **9h.** Apparently electron-withdrawing groups in the 5- and 7-positions may not be critical for the potency of HNQs and QTOs, while electron-withdrawing groups in those positions are important for the potency of QXs.

It is also noteworthy that 6,7,8-trisubstituted (**8j**) and 5,6,7,8-tetrasubstituted (**8k**) HNQs are much less active than the corresponding 6,7,8-trisubstituted (**9j**) and 5,6,7,8-tetrasubstituted (**9k**) QXs. Two factors could contribute to the observed difference in potency. First, the Cl and F in **9j**,**k** serve as electron-withdrawing groups, increasing the potency of the QXs. Second, QXs are symmetrical with respect to the two amide NHs, both being potentially available for hydrogen-bonding depending on the orientation of the ligand in the receptor. In contrast, the F atom in the 8-position of **8j**,**k** might serve to reduce the potency of HNQs, such

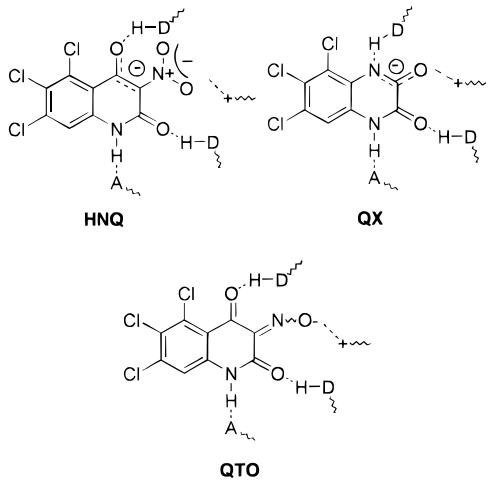


Figure 1. Glycine site binding model for HNQs in comparison with QXs and QTOs. A represents a receptor hydrogen bond acceptor and D−H represent a receptor hydrogen bond donor; + represents a positively charged receptor group.

as by interfering with the critical hydrogen-bonding of the NH in the 1-position.

Comparing the SAR of HNQs **8** with that of QTOs **10** reveals that for compounds with the same substituents, HNQs are generally about 10 times less potent than QTOs. Interestingly, both **8e** and **10e** are inactive, lending further support to the notion that an unhindered NH in the 1-position of the HNQs and QTOs is important for interaction with the receptor.

The similarity among the SARs of HNQs **8**, QXs **9**, and QTOs **10** suggests that these three classes of ligands may bind to the glycine site of NMDA receptors in a similar way. We propose the following model for HNQs binding to the glycine site of NMDA receptors which is similar to the models proposed for QXs and QTOs[11] (Figure 1) and also for 3-acyl-4-hydroxyquinolin-2(1*H*)-ones.[8] The model consists of a hydrogen bond donor (NH) in the 1-position, a hydrogen bond acceptor (C=O) in the 2-position, a charge−charge interaction in the 3- to 4-positions, a hydrogen bond acceptor in the 4-position, and hydrophobic interactions involving the benzene ring portion of the molecules. Since HNQs are more acidic than QXs,[15] presumably owing to ionization of the 4-OH group, electron-withdrawing groups in the benzene ring are not critical for the potency of HNQs, though they are very important for the potency of QXs. Similarly, electron-withdrawing groups in the benzene ring are not critical for potency of QTOs.[11] HNQs in general are less active than QTOs, possibly because there is better charge−charge interaction between QTOs and the receptor than that of HNQs. As seen from the model, the negative charge in HNQs is distributed over several atoms, while the negative charge in QTOs is likely to be more directional and is located mainly on the N−O oxygen.

Functional antagonism of NMDA and AMPA receptors by selected HNQs is summarized in Table 2. The glycine K_b values obtained from electrophysiology for **8f**,**h** are about 2- and 3-fold higher (i.e., less potent) than the corresponding IC_{50} values obtained from [^{3}H]DCKA binding, while the K_b value for **8i** indicates that this compound is about 3-fold more potent in the functional assay. Based on the electrophysiology assays, 5,6,7-trichloro HNQ (**8i)** is about 5-fold more potent than 5,7-dichloro HNQ (**8f)** and about equipotent to the corresponding 5,6,7-trichloro QX (**9i**). This is reasonable given the similar SAR of HNQs and QXs. The moderate differences observed between IC_{50} values from [^{3}H]-DCKA binding and K_b values from electrophysiology could be due to several factors including differences in affinity between cloned 1a/2C NMDA receptors and the heterogeneous native NMDA receptor population present in rat brain cortical membranes.

In a second series of electrophysiological experiments, we assayed effects of **8i** on glycine concentration−response curves in oocytes expressing the NR 1a/2C receptors. As shown in Figure 2, 1 μM **8i** shifted the affinity for glycine from 170 nM to 3.0 μM, an 18-fold increase. The K_b for **8i** at the NMDA receptor glycine site was assessed by simultaneous fits of glycine inhibition curves alone and in the presence of 1 μM **8i**. Analysis of these data gives a K_b value for **8i** of 0.058 (0.056−0.059) μM, which is close to that estimated from the inhibition curve (0.079 μM). Statistical analysis of the curve shift induced by 1 μM **8i** indicates that inhibition conforms to the competitive model ($F_{1,38}$ = 0.30).

Although 10 μM **8i** shifts the glycine curve further to the right, inhibition at this concentration of antagonist is not fully surmountable. This suggests that high concentrations of **8i** may induce inhibition that is not completely due to glycine site antagonism. No further experiments were performed to investigate this effect. Due to the insurmountable inhibition at 10 μM **8i**, statistical analysis performed using the control, 1 and 10 μM **8i** curves did not conform to the competitive model ($F_{2,57}$ = 21.7).

The three HNQs tested all have low affinity for AMPA receptors (Table 2). Interestingly, AMPA receptor potency almost directly parallels glycine site potency, so there is only a 2-fold difference in selectivity for these three HNQs. HNQ **8i** is the most selective ligand with a selectivity of 240-fold favoring the NMDA receptor glycine site. The other two HNQs also have a selectivity of >100-fold, suggesting that HNQs generally are highly selective for the glycine site. By comparison, QX **9i** shows a 50-fold selectivity, and only the most potent compounds in the QX series such as ACEA 1021 (**5)** have a selectivity of >100-fold.[10] The most selective compound among the QTOs (**10i)** has a selectivity of 80-fold.[11]

The most striking structural difference between HNQs and the broad spectrum antagonist **4** is the 4-OH group. The low AMPA activity observed for the HNQs is consistent with the pharmacophore model proposed by Carling et al. for AMPA receptors.[9] They propose that AMPA receptors exhibit a bulk intolerance adjacent to the 4-position, while for the glycine site a hydrogen-bond-accepting group adjacent to the 4-position is obligatory for good potency.[9] Therefore, the presence of the 4-OH group in the HNQs appears to favor the glycine site of NMDA receptors and disfavor the glutamate site of AMPA receptors, thus accounting for their high selectivity.

Conclusions

In conclusion, a series of HNQs has been synthesized and found to be a novel class of potent and selective antagonists for the glycine site of NMDA receptors. Substitution in the benzene portion of HNQ by chloro

Table 2. Functional Antagonism by HNQs of NR 1a/2C NMDA Receptors and Rat Brain AMPA Receptors Expressed in *Xenopus* Oocytes

	8				9[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)
f	0.41 (0.39−0.44)[f]	37 (33−41)	118	3, 3	0.73	9.3
h	0.91 (0.86−0.95)	150 (140−170)	164	3, 3	ND[g]	ND
i	0.079 (0.072−0.087)	19 (17−20)	240	3, 3	0.082	4.2

[a] Inhibition of NMDA receptors was measured in oocytes expressing the cloned rat receptor subunit combination NR 1a/2C. K_b values for 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 for glutamate binding sites were estimated from inhibition of currents elicited by 10 μM AMPA. HNQs were initially dissolved in DMSO, and the final aqueous solution contained 0.1−1% DMSO. [c] The steady-state selectivity index for inhibition at NMDA receptor glycine sites was estimated by dividing K_b AMPA by K_b glycine. [d] Indicates the number of independent experiments (cells examined); numbers refer to NMDA and AMPA, respectively. [e] Data from ref 10 for QXs. [f] Numbers in parentheses are 95% confidence intervals adjusted to the linear scale. [g] ND, not determined.

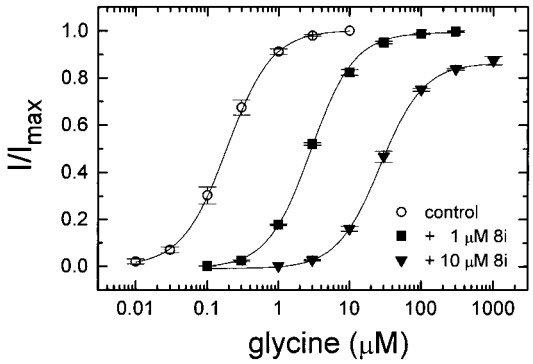


Figure 2. Inhibition of NR 1a/2C receptors in *Xenopus* oocytes by **8i**. Inhibition is associated with a rightward shift in the glycine concentration−response relationship. Curves were first measured under control conditions and then repeated in two concentrations of **8i**. A fixed concentration of 100 μM glutamate was used throughout. The EC_{50} and slope values for the three curves measured independently were as follows: control, 0.17 μM and 1.4; 1 μM **8i**, 3.0 μM and 1.4; 10 μM **8i**, 40 μM and 1.0. Amplitudes of maximal responses ranged between 61 and 535 nA (mean = 280 ± 173 (n = 3)).

and methyl, especially in the 5-, 6-, and 7-positions, was found to increase the potency of the HNQs. 5,6,7-Trichloro HNQ (**8i**) is the most potent antagonist with an IC_{50} of 220 nM in DCKA binding and a K_b of 79 nM in electrophysiological assays. HNQ **8i** is 240-fold selective for NMDA vs AMPA receptors. Comparison of the SAR of HNQ with the SAR of QX and QTO suggests that these three classes of antagonists most probably bind to the glycine site in a similar manner.

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 a VG ZAB-2FHF mass spectrometer. The preparation of 2,4-quinolinediols **7b**−**k** has been reported.[11]

6,7,8-Trichloro-2,4-quinolinediol (7l). To a solution of 2,3,4-trichloroaniline (985 mg, 5.0 mmol) and Et_3N (714 mg, 7.05 mmol) in anhydrous ether (80 mL) was added dropwise a solution of ethyl malonyl chloride (900 mg, 6.0 mmol) in dry ether (15 mL) at room temperature. The resulting mixture was stirred at room temperature under N_2 for 20 h. The reaction mixture was diluted with H_2O (50 mL) and then acidified to pH = 2 with 2 N HCl. The ether layer was separated, and the aqueous phase was extracted with ether (2 × 25 mL). The ether solutions were combined, washed with brine, dried (Na_2SO_4), and evaporated to dryness. To the residue were added MeOH (50 mL) and 1 N NaOH (20 mL). The resulting mixture was refluxed for 3 h, cooled to room temperature, and acidified to pH = 2 with 2 N HCl. The mixture was evaporated, and the residue was extracted with $CHCl_3$ (3 × 30 mL). The $CHCl_3$ solutions were combined, washed with brine, dried (Na_2SO_4), and evaporated to give 525 mg (37%) of the acid as a yellowish powder: mp 160−161 °C; ^{1}H NMR (DMSO-d_6) 3.50 (s, 2H), 7.64 (d, J = 9, 1H), 7.87 (d, J = 9, 1H), 10.06 (s, 1H).

A mixture of the acid (490 mg, 1.74 mmol) with polyphosphoric acid (20 g) was stirred at 170 °C for 3 h and then cooled to room temperature. To the resulting mixture were added slowly 150 mL of 1 N HCl and then 20% of aqueous NaOH to adjust the pH to 4. The precipitate was filtered, washed with H_2O (5 × 5 mL), and dried to give 270 mg of crude product. The crude product was treated with DMSO (20 mL) and filtered. To the filtrate was added water (100 mL), and the precipitate was filtered, washed with water (3 × 5 mL), and dried to give 230 mg (50%) of **7l** as a cream powder: mp 325 °C dec; ^{1}H NMR (DMSO-d_6) 5.83 (s, 1H), 7.91 (s, 1H), 10.77 (s, 1H), 11.98 (s, 1H).

5,7-Dichloro-4-hydroxy-3-nitroquinolin-2(1*H*)-one (8f). To a mixture of 5,7-dichloro-1,4-quinolinediol (**7f**) (534 mg, 2.32 mmol) in glacial acetic acid (3 mL) was added HNO_3 (69−71%, 0.7 mL), and the mixture was heated at 90 °C for 30 min. The mixture was cooled to room temperature, filtered, washed with water, and dried to leave 562 mg (88%) of **8f** as a yellow solid: mp 170−171 °C dec; ^{1}H NMR ($CDCl_3$ + DMSO-d_6) 6.91 (d, J = 1.8, 1H), 7.00 (d, J = 1.8, 1H), 11.99 (sb, 1H); HRMS calcd for $C_9H_4{}^{35}Cl_2N_2O_4$ 273.9544, found 273.9551. Anal. ($C_9H_4Cl_2N_2O_4$) C, H; N: calcd, 10.18; found, 9.75.

HNQs **8a**−**e,h,j,k** were prepared from the corresponding 2,4-quinolinediols using the same procedures.

4-Hydroxy-3-nitroquinolin-2(1*H*)-one (8a): mp 216 °C dec (lit.[12] mp 216 °C); ^{1}H NMR ($CDCl_3$ + DMSO-d_6) 7.01 (t, J = 7.5, 1H), 7.11 (d, J = 7.5, 1H), 7.40 (t, J = 7.2, 1H), 7.88 (d, J = 7.8, 1H), 11.72 (mb, 1H).

7-Chloro-4-hydroxy-3-nitroquinolin-2(1*H*)-one (8b): mp 220−222 °C dec; ^{1}H NMR (DMSO-d_6) 7.21 (m, 2H), 7.94 (d, J = 7.4, 1H), 11.69 (s, 1H); HRMS calcd for $C_9H_5{}^{35}ClN_2O_4$ 239.9938, found 239.9946. Anal. ($C_9H_5ClN_2O_4$) C, H, N.

6-Chloro-4-hydroxy-3-nitroquinolin-2(1*H*)-one (8c): mp 223−225 °C dec (lit.[12] mp 200 °C); ^{1}H NMR (DMSO-d_6) 7.21 (d, J = 8.7, 1H), 7.63 (d, J = 8.7, 1H), 7.96 (s, 1H), 11.80 (s, 1H); HRMS calcd for $C_9H_5{}^{35}ClN_2O_4$ 239.9938, found 239.9929.

5-Chloro-4-hydroxy-3-nitroquinolin-2(1*H*)-one (8d): mp 180−182 °C dec; ^{1}H NMR (DMSO-d_6) 7.20 (m, 2H), 7.46 (m, 1H), 11.73 (s, 1H); HRMS calcd for $C_9H_5{}^{35}ClN_2O_4$ 239.9938, found 239.9929. Anal. ($C_9H_5ClN_2O_4$) C, H, N.

8-Chloro-4-hydroxy-3-nitroquinolin-2(1*H*)-one (8e): mp 205−206 °C (lit.[12] mp 204−205 °C); ^{1}H NMR (DMSO-d_6) 7.21 (dd, J = 8.1, 7.5, 1H), 7.71 (d, J = 7.5, 1H), 7.95 (d, J = 8.1, 1H), 10.87 (bs, 1H); HRMS calcd for $C_9H_5{}^{35}ClN_2O_4$ 239.9938, found 239.9934.

5,7-Dimethyl-4-hydroxy-3-nitroquinolin-2(1*H*)-one (8h): mp 188−190 °C (lit.[12] mp 226 °C); ^{1}H NMR (DMSO-d_6) 2.28 (s, 3H), 2.66 (s, 3H), 6.61 (s, 1H), 6.81 (s, 1H), 11.52 (s, 1H); HRMS calcd for $C_{11}H_{10}N_2O_4$ 234.0639, found 234.0651.

7-Chloro-6,8-difluoro-4-hydroxy-3-nitroquinolin-2(1*H*)-one (8j): mp 200−201 °C dec; ^{1}H NMR (DMSO-d_6) 7.66 (d, J = 9.3, 1H), 11.07 (mb, 1H); HRMS calcd for $C_9H_3{}^{35}ClF_2N_2O_4$ 275.9746, found 275.9741. Anal. ($C_9H_3ClF_2N_2O_4$) C, H; N: calcd, 10.13; found, 9.67.

5,6,7,8-Tetrafluoro-4-hydroxy-3-nitroquinolin-2(1*H*)-one (8k): mp 176−177 °C dec; ^{1}H NMR (DMSO-d_6) 10.80 (mb, 1); HRMS calcd for $C_9H_2F_4N_2O_4$ 277.9948, found 277.9970. Anal. ($C_9H_2F_4N_2O_4$) C, H, N.

6,7-Dichloro-4-hydroxy-3-nitroquinolin-2(1*H*)-one (8g). To a mixture of 6,7-dichloro-2,4-quinolinediol and 5,6-dichloro-2,4-quinolinediol **(**1:1**)**[11] (1.25 g, 5.43 mmol) in glacial acetic acid (8 mL) was added HNO_3 (69−71%, 1.5 mL), and the mixture was heated at 90 °C for 2 h. The mixture was cooled to room temperature, filtered, washed with water, and dried to leave 280 mg (19%) of **8g** as a yellow solid: mp 235−236 °C dec; ^{1}H NMR ($CDCl_3$ + DMSO-d_6) 7.36 (s, 1H), 8.04 (s, 1H), 11.37 (s, 1H); HRMS calcd for $C_9H_4{}^{35}Cl_2N_2O_4$ 273.9544, found 273.9548. Anal. ($C_9H_4Cl_2N_2O_4$) C, H; N: calcd, 10.18; found, 9.76.

5,6,7-Trichloro-4-hydroxy-3-nitroquinolin-2(1*H*)-one (8i). A mixture of 5,6,7-trichloro-2,4-quinolinediol (**7i**) (264 mg, 1.0 mmol) and KNO_3 (120 mg, 1.2 mmol) in trifluoroacetic acid (5 mL) was refluxed for 24 h, cooled to room temperature, and diluted with water (20 mL). The precipitate was filtered, washed with water (3 × 2 mL) and ethanol (2 × 2 mL), and dried to give 120 mg (39%) of **8i** as a yellow powder: mp 225 °C dec; ^{1}H NMR (DMSO-d_6) 7.54 (s, 1H), 10.93 (bs, 1H). Anal. ($C_9H_3Cl_3N_2O_4 \cdot 0.2H_2O$) C, H, N.

6,7,8-Trichloro-4-hydroxy-3-nitroquinolin-2(1*H*)-one (8l). HNQ **8l** was prepared from **7l** in a manner similar to **8i** as an orange-yellow powder: mp 222−224 °C; ^{1}H NMR (DMSO-d_6) 8.01 (s, 1H), 9.96 (bs, 1H). Anal. ($C_9H_3Cl_3N_2O_4 \cdot 0.2H_2O$) C, H; N: calcd, 8.94; found, 8.22.

Pharmacology. DCKA Binding Assay. The [^{3}H]DCKA binding assay was performed according to the method reported in ref 11 for QTOs.

Electrophysiology. Electrophysiological assay of selected HNQs on NMDA and AMPA receptors was carried out according to the method reported in ref 11.

Acknowledgment. We thank Dr. Yan Ni and Dr. Ricardo Miledi (University of California, Irvine) for the generous gift of rat cerebral cortex poly(A)$^+$ RNA used in this study, Dr. P. H. Seeburg (Heidelburg University, Heidelburg, Germany) for the cDNAs encoding rat NR1 and NR2 subunits, and Dr. John Guastella (CoCensys, Inc.) for preparation of cRNAs encoding NMDA receptor subunits. We also thank Dr. Yixin Lu of the University of Oregon for preparation of HNQ **8h.** Financial support was provided in part by CoCensys, Inc., and by the National Institute of Drug Abuse (DA-06726).

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JM960520Y