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1-HYDROXY-1,4-DIHYDROQUINOXALINE-2,3-DIONES: NOVEL ANTAGONISTS AT NMDA RECEPTOR GLYCINE SITES

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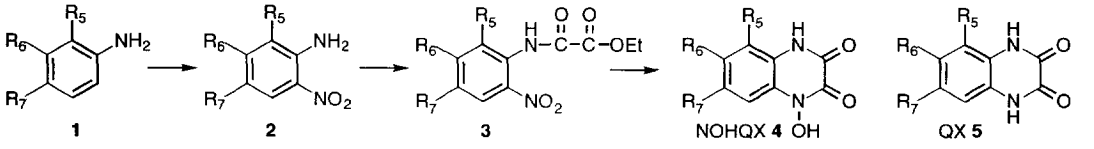
Abstract: A series of 1-hydroxy-1,4-dihydroquinoxaline-2,3-diones was synthesized and assayed for NMDA receptor glycine site antagonism. Except for **4a**, these were found to be 3–80 times weaker antagonists than the analogous 1,4-dihydroquinoxaline-2,3-diones.

Certain substituted 1,4-dihydroquinoxaline-2,3-diones (QXs) are potent antagonists[1] at *N*-methyl-D-aspartate (NMDA) receptor glycine sites[2] and have been shown to be neuroprotective in animal models of ischemic stroke.[3] The amide functional group found in the QXs and many other glycine site antagonists[4] appears to play an important role in receptor binding via hydrogen bonding.[2] QXs substituted with combinations of electron withdrawing groups such as bromine, chlorine, cyano or nitro are particularly potent. These substituents probably increase the acidity of the amide hydrogen. This suggests that replacement of the amide NH (pKa ~17)[5] with a more acidic group such as a *N*-hydroxyamide (pKa ~9)[6] may yield antagonists with superior potency. To test this hypothesis, we prepared a series of 1-hydroxy-1,4-dihydroquinoxaline-2,3-diones (NOHQXs) and compared their potency as antagonists at NMDA glycine sites to the potency of analogous QXs.

The synthesis of NOHQXs followed the general method of Loev et al.[7] Commercially available *o*-nitroanilines **2** were allowed to react with ethyl oxalyl chloride yielding the corresponding oxamates **3** (60-82%). Partial hydrogenation (Pd/C, DMF) of the nitro group to the *N*-hydroxyl amine with concomitant cyclization led directly to the desired NOHQXs **4** (51-93%). A variation on this method was employed when appropriately substituted *o*-nitroanilines were not commercially available. For example, treatment of 4-trifluoromethylaniline (**1**, $R_5 = R_6 = H$; $R_7 = CF_3$) with 1.1 equiv of *N*-chlorosuccinimide in DMF[8] followed by reaction with ethyl oxalyl chloride and subsequent nitration (KNO_3 in H_2SO_4)[9] gave 2-nitrooxamate **3** ($R_5 = Cl$; $R_6 = H$; $R_7 = CF_3$). Reduction and cyclization as above yielded NOHQX **4d** (26% overall).



1 **2** **3** NOHQX **4** OH QX **5**

Potencies for NOHQXs **4a-h**[10] and the QXs **5a-h**[1] are listed in Table 1. The data were obtained using a [^{3}H]5,7-dichlorokynurenic acid (DCKA) binding assay[11] and an electrophysiological assay in *Xenopus* oocytes.[1] Potency in the DCKA assay is indicative of binding at NMDA glycine sites. The electrophysiological assay indicates that the NOHQXs are functional NMDA receptor antagonists. The first NOHQX examined, dichloro NOHQX **4a**, was more potent than the corresponding QX **5a**. This promising observation did not endure, however. NOHQX **4a** has its amide NH group adjacent to an H atom at C5. All the other NOHQXs

have a non-hydrogen substituent attached to C5. These latter NOHQXs are substantially less potent (3 to 80-fold) than the analogous QXs. We postulate that the receptor prefers to H-bond to NH over NOH. The C5 substituent on the aromatic ring peri to the lone NH group in NOHQXs **4b-h** probably inhibits hydrogen bonding to the receptor.[12] QXs, on the other hand, are symmetrical in the heterocyclic portion and therefore can become positioned within the receptor so that the less hindered amide is available for hydrogen bonding.

Table 1. IC_{50}[a] and K_b[b] Values

Cpd. No.	R_5	R_6	R_7	NOHQXs 4		QXs 5	
				K_b (μM) (95% Confidence)	IC_{50} (μM)	K_b (μM)	IC_{50} (μM)
a	H	Cl	Cl	0.042 (0.033-0.054)	0.081±0.015	0.38	0.13±0.03
b	NH_2	Cl	Cl	0.44 (0.39-0.50)	1.8±0.2	0.059	0.093±0.014
c	Br	Cl	Cl	1.2 (0.9-1.6)	2.7±0.1	0.095	0.21±0.03
d	Cl	H	CF_3	1.6 (1.3-2.0)	25±1	0.52	2.1±0.1
e	Cl	Cl	Cl	1.6 (1.2-2.0)	2.5±0.2	0.082	0.030±0.005
f	Cl	H	Cl	10 (8.5-12)	19±3	0.73	0.28±0.02
g	Br	H	CF_3	>10	54±11	1.0	2.5±0.5
h	Br	H	Br	>10	20±1	0.89	1.3±0.2

a. IC_{50} values were determined in rat brain cortical membranes using a [^{3}H]DCKA binding assay as described in reference 11. Values are the mean ± SEM of at least three independent experiments. *b*. K_b values for NOHQXs were determined by electrophysiological assays in *Xenopus* oocytes expressing cloned NMDA receptors [NR1A/2C] as described in reference 1. Values are the mean from 3-4 separate measurements, except for **4g** and **4h** where two measurements were made. K_b values for QXs are those reported in reference 1 and are included for purposes of comparison.

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10. All intermediates and final compounds gave satisfactory 300 MHz ^{1}H NMR spectra. **4a**: mp 300 °C (lit.[13] mp dec), CHN; **4b**: mp 300 °C dec, found m/e 260.9717; **4c**: mp 276-278 °C, found m/e 323.8685; **4d**: mp 283-286 °C, CHN; **4e**: mp 350 °C dec, CHN; **4f**: mp 285-288 °C, CHN; **4g**: mp 300 °C dec, found m/e 323.9358; **4h**: mp 285-287 °C, found m/e 333.8610.
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