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Synthesis and Excitatory Amino Acid Pharmacology of Some Novel Quinoxalinediones

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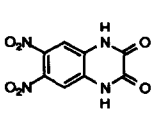
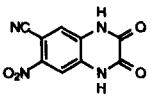
Abstract: The synthesis and amino acid pharmacology of twelve N-substituted quinoxalinediones is reported. In particular, compounds **4a** and **4b** show significant antagonism at both the AMPA and glycine-site NMDA receptors. The functional antagonism of **4a** has been demonstrated.

The excitatory amino acid neurotransmitter glutamate is thought to be involved with several neurodegenerative and neuropathological disorders, including ischemia,[3] epilepsy,[4] Huntington's disease,[5] and Alzheimer's disease.[6] Consequently, there is much interest in the modulation of glutamate receptors, especially the N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA) subtypes.[7] Additionally, the NMDA receptor also contains a requisite glycine modulatory site[8] for which selective antagonists block the NMDA receptor.[9]

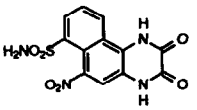
Quinoxalinediones such as 6,7-dinitroquinoxaline-2,3-dione (**1**, DNQX), and 6-cyano-7-nitroquinoxaline-2,3-dione (**2**, CNQX) have been shown to be AMPA[10] as well as glycine antagonists,[11] and also to be neuroprotective in vitro.[12] In addition, the AMPA selective quinoxalinedione 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(F)quinoxaline (**3**, NBQX) has been shown to be neuroprotective in cerebral ischemia models.[13]



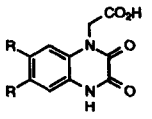
1
DNQX



2
CNQX



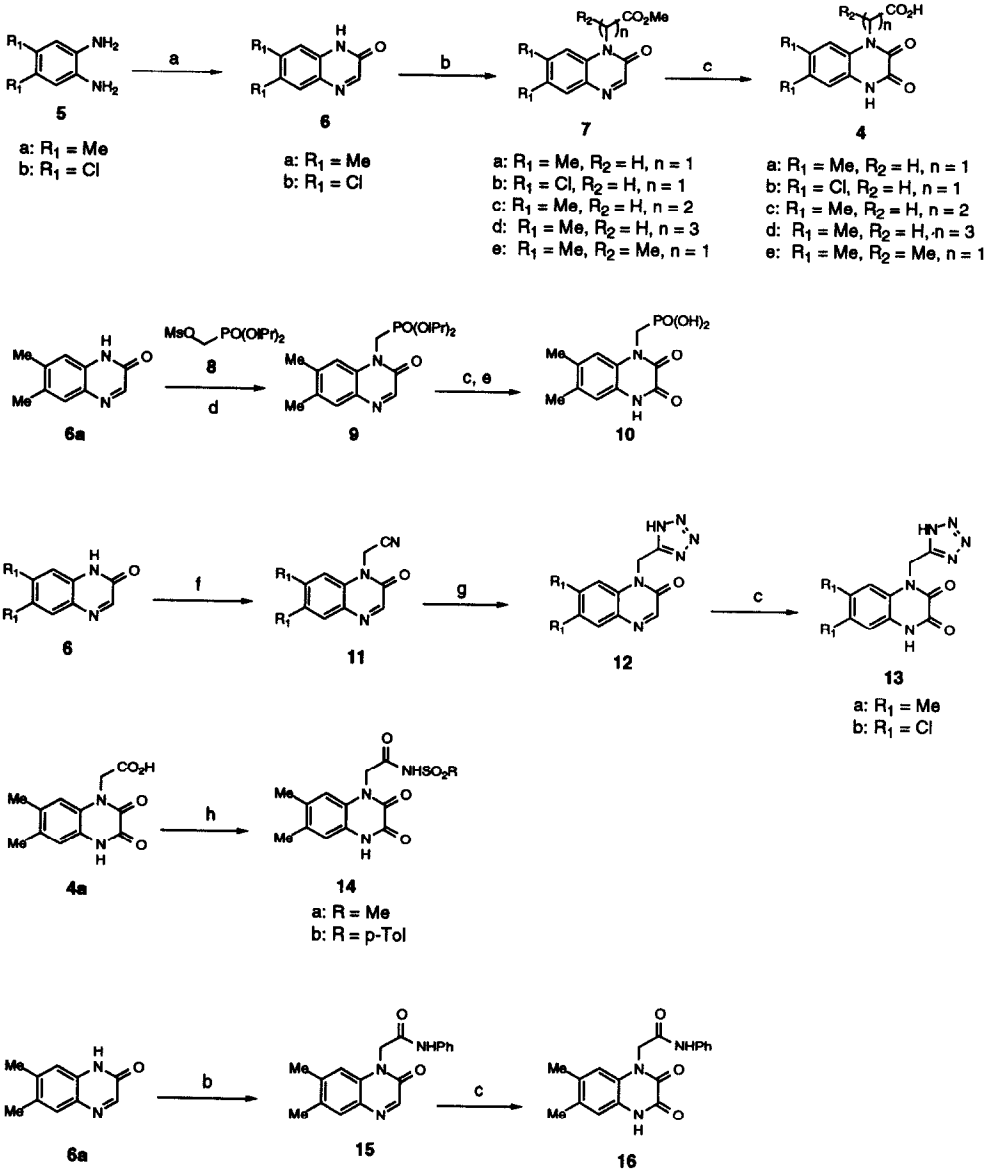
3
NBQX



4a R = CH_3
4b R = Cl

In contrast to these quinoxalinediones, some other classes of glycine and AMPA antagonists explore an additional carboxylate binding site. In particular, 3-substituted 2-carboxyindoles[14] and 4-substituted 2-carboxytetrahydroquinolines[15] have been shown to be potent glycine antagonists. We therefore initiated a program to construct quinoxalinediones with functional group-containing side chains to investigate this acidic binding site. We now report a series of twelve quinoxalinediones functionalized at N-1, exemplified by acids **4a** and **4b**, that are potent AMPA/glycine antagonists. These compounds fall into two broad groups relative to **4**: (1) acid isosteres or replacements, and (2) chain extended acids or α-substituted acids.

Scheme 1. Synthesis of quinoxalinediones.



(a) $OHCCO_2Bu$, EtOH; (b) NaH or LDA, RX, DMF; (c) 30% H_2O_2, AcOH; (d) **8**, Cs_2CO_3, DMF; (e) 48% HBr, AcOH; (f) $BrCH_2CN$, NaH, DMF; (g) Bu_3SnN_3, toluene,140 °C; (h) RSO_2NH_2, DPPA, DBU, DMSO.

Table 1. Displacement of [^{3}H]AMPA and [^{3}H]glycine by quinoxalinediones.

compound	[^{3}H]AMPA K_i (μM) (% displacement of [^{3}H]AMPA at 10 μM)	[^{3}H]glycine K_i (μM) (% displacement of [^{3}H]glycine at 10 μM)
4a	0.70	13.8
4b	2.10	0.11
4d	8.0	(29.6)
10	1.21	5.28
13b	(58.6)	(58.8)
4c	(28.5)	(58.2)
13a	(52.4)	(49.6)
14a	(49.0)	(36.0)
14b	(28.0)	(48.0)
4e	(13.9)	(2.6)
7a	(4.6)	(8.0)
16	(5.2)	(2.9)

CNQX was used as an AMPA standard and exhibited a Ki = 0.36 μM vs. [^{3}H]AMPA. DCKA was used as a standard for glycine and exhibited a Ki = 0.50 μM vs. [^{3}H]glycine. K_i values represent duplicate determinations.

Phenylenediamine **5** was condensed with n-butyl glyoxalate to provide quinoxalinone **6**,[16] which was alkylated under a variety of conditions. Acids **4a-e** were prepared by alkylating **6** with the appropriate bromo ester (NaH or LDA, DMF) to give **7** followed by saponification (1 N NaOH, EtOH) and oxidation to the quinoxalinedione (30% H_2O_2, AcOH). Phosphonate **10** was prepared by alkylating **6a** with mesylate **8**[17] (Cs_2CO_3, DMF) followed by oxidation and hydrolysis of the phosphonate ester **9** (48% HBr, AcOH). Tetrazoles **13a** and **13b** were prepared by first alkylating **6** with bromoacetonitrile to provide **11** and then converting the nitrile to the tetrazole[18] (Bu_3SnN_3, toluene, 140 °C) followed by oxidation to the quinoxalinedione. Acid **4a** was converted to the sulfonimides **14a** and **14b** by treatment of **4a** with either methyl- or p-toluenesulfonamide[19] (DPPA, DBU, DMSO). Amide **16** was prepared by alkylating **6a** with N-phenyl-2-bromoacetamide followed by oxidation.

The quinoxalinediones were evaluated for their abilities to displace either [^{3}H]AMPA[20] or [^{3}H]glycine[21] in rat cortical membranes (Table 1). K_i values were determined for those compounds which demonstrated greater than 60% displacement at 10 μM. In general, the acetic acid side chain or acid isosteres showed the greatest affinity. The dichloro compound **4b** exhibited the greatest affinity for the glycine site with a K_i value of 110 nM and was less potent at the AMPA receptor with a K_i value of 2.10 μM. Compound **4a** showed the greatest AMPA affinity with a K_i value of 700 nM, but was less active at the glycine site where the K_i value increased to 13.8 μM. Phosphonate **10** showed balanced affinity at both AMPA receptor (1.21 μM) and glycine site (5.28 μM). Tetrazoles **13a** and **13b** were moderately active as were sulfonates **14a** and **14b**.

Increasing the chain length of acid **4** diminished activity. Interestingly, the butanoate side chain of **4d** decreased glycine antagonism more than AMPA antagonism, while the propionate side chain of **4c** had the opposite effect, decreasing AMPA antagonism more than glycine antagonism. Replacing the acid moiety

of **4** with either an ester (**7a**) or an amide (**16**) resulted in loss of activity as did α-substitution of the acid (**4e**). The diminished activity of ester **7a** to acid **4a** is in direct contrast to glycine-site pharmacology of 4-substituted 2-carboxyindoles. Likewise, the diminished activity of amide **16** to acid **4a** contrasts to the glycine-site pharmacology of 4-substituted 2-carboxytetrahydroquinolines.

The functional antagonism of excitatory amino acid-mediated neurotransmission by **4a** was demonstrated on rat hippocampal slices.[22] When the response to electrical stimulation of the Schaffer-collateral commissural fiber tract (an excitatory amino acid pathway) was recorded in the cell body layer or apical dendritic field of area CA_1, compound **4a** reversibly depressed both population spike amplitude and excitatory post-synaptic potentials (EPSP) respectively at concentrations of 10, 50, and 100 μM, with an estimated $EC_{50} = 14.53$ μM for EPSP slope depression. CNQX[23] had an estimated $EC_{50} = 2.74$ μM in the same experiment. The population spike, which reflects the cellular response to the EPSP, was completely suppressed by **4a** at 10 μM.

In conclusion, twelve quinoxalinediones were synthesized as potential excitatory amino acid antagonists. Of these compounds, quinoxalinediones containing acetic acid side chains demonstrated the greatest affinity for both the AMPA and glycine-site NMDA receptors, although phosphonic acid **10** also showed high affinity for both receptors. Compound **4a** demonstrated functional antagonism in a rat brain preparation. It is noteworthy that the functional groups in the side chains of these quinoxalinediones did not parallel the SAR of known glycine antagonists.

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