Articles

Kynurenic Acid Derivatives. Structure–Activity Relationships for Excitatory Amino Acid Antagonism and Identification of Potent and Selective Antagonists at the Glycine Site on the *N*-Methyl-D-aspartate Receptor

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Derivatives of the nonselective excitatory amino acid antagonist kynurenic acid (4-oxo-1,4-dihydroquinoline-2-carboxylic acid, **1**) have been synthesized and evaluated for in vitro antagonist activity at the excitatory amino acid receptors sensitive to *N*-methyl-D-aspartic acid (NMDA), quisqualic acid (QUIS or AMPA), and kainic acid (KA). Introduction of substituents at the 5-, 7-, and 5,7-positions resulted in analogues having selective NMDA antagonist action, as a result of blockade of the glycine modulatory (or coagonist) site on the NMDA receptor. Regression analysis suggested a requirement for optimally sized, hydrophobic 5- and 7-substituents, with bulk tolerance being greater at the 5-position. Optimization led to the 5-iodo-7-chloro derivative (**53**), which is the most potent and selective glycine/NMDA antagonist to date (IC_{50} vs [^{3}H]glycine binding, 32 nM; IC_{50}'s for other excitatory amino acid receptor sites, >100 μM). Substitution of **1** at the 6-position resulted in compounds having selective non-NMDA antagonism and 8-substituted compounds were inactive at all receptors. The retention of glycine/NMDA antagonist activity in heterocyclic ring modified analogues, such as the oxanilide **69** and the 2-carboxybenzimidazole **70**, suggests that the 4-oxo tautomer of **1** and its derivatives is required for activity. Structurally related quinoxaline-2,3-diones are also glycine/NMDA antagonists, but are not selective and are less potent than the **1** derivatives, and additionally show different structure–activity requirements for aromatic ring substitution. On the basis of these results, a model accounting for glycine receptor binding of the **1** derived antagonists is proposed, comprising (a) size-limited, hydrophobic binding of the benzene ring, (b) hydrogen-bond acceptance by the 4-oxo group, (c) hydrogen-bond donation by the 1-amino group, and (d) a Coulombic attraction of the 2-carboxylate. The model can also account for the binding of quinoxaline-2,3-diones, quinoxalic acids, and 2-carboxybenzimidazoles.

There is increasing evidence that abnormal activation of excitatory amino acid receptors[1] may be associated with several disorders of the central nervous system. In particular excessive stimulation of the postsynaptic receptor sensitive to *N*-methyl-D-aspartic acid (NMDA)[2] has been implicated as a significant contributory mechanism to neuronal death in cerebral ischemia, epilepsy, and Alzheimer's disease. Competitive and uncompetitive antagonists acting at the NMDA receptor may be of therapeutic benefit, since these compounds have been shown to be neuroprotective and anticonvulsant in a variety of animal models. Competitive antagonists, for example [3-(2-carboxypiperazin-4-yl)propyl]phosphonic acid[3] (CPP) and related amino acid phosphonates,[4-6] act at the neurotransmitter (probably glutamate) recognition site. Uncompetitive antagonists, which include (+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine[7] (MK-801) and analogues,[8-10] block the open (activated) state of the cation-permeable channel associated with the NMDA receptor. However both competitive NMDA antagonists and channel blockers produce in vivo behavioral effects that may limit their utility.[11]

Recently it has been demonstrated that the amino acid glycine[12] amplifies the agonist action of NMDA, by a mechanism not involving the established strychnine-sensitive glycine receptor. The presence of glycine appears to be obligatory for NMDA-receptor action, and the accumulating evidence indicates that glycine acts as an endogenous coagonist[13] at a specific site on the NMDA receptor (the glycine/NMDA site). These findings have led to an intensive search for alternative NMDA antagonists that inhibit this action of glycine.[14-24] Compounds re-

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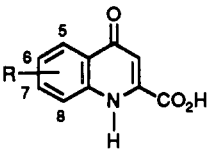
Table I. Chloro-Substituted Kynurenic Acids

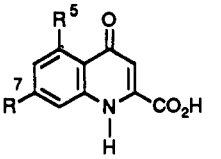


no.	R	[^{3}H]Gly IC_{50},[a] μM	Kb,[b] μM NMDA	QUIS	KA
1		41	154	120	89
10	5-Cl	5.2	37	97	>100
11	6-Cl	>100	>100	30	19
2	7-Cl	0.56	7.0	63	50
12	8-Cl	>100	IA@100	IA@100	IA@100
13	5,7-Cl_2	0.20	3.0	>30	>30
14	5,6,7-Cl_3	2.4	28	15	14

[a] Inhibition of [^{3}H]glycine binding to rat cortical membranes. [b] Antagonism of depolarizations due to agonists in a rat cortical slice preparation. NMDA, *N*-methyl-D-aspartic acid; QUIS, quisqualic acid; KA; kainic acid. IA = inactive at stated concentration; > = dose ratio less than 2 at stated concentration. See text and refs 15 and 19 for details of the assay procedures.

ported to possess glycine/NMDA antagonist activity include the benzo-fused heterocycles kynurenic acid (1),[14] 7-chlorokynurenic acid (2),[15] quinoxaline-2,3-diones[16] (e.g. the 6,7-dinitro derivative 3), and 6,7-dichloroquinoxalic acid (4).[17] These compounds insurmountably block NMDA responses, and probably act as pure antagonists (or possibly as inverse agonists) at the glycine site. In contrast, certain cyclic amino acid derivatives such as 5[18-20]

Table II. 5- and 7-Substituted Kynurenic Acids



no.	R^5	R^7	[^{3}H]Gly IC_{50},[a] μM	NMDA Kb,[b] μM
1	H	H	41	154
15	F	H		41
10	Cl	H	5.2	37
16	Br	H		20
17	I	H	0.63	7.6
18	CF_3	H		35
19	CH_3	H	2.1	21
20	C_2H_5	H	4.8	5.5
21	$CH{=}CH_2$	H	>100	>300
22	*n*-C_3H_7	H		400
23	*i*-C_3H_7	H		>100
24	*n*-C_4H_9	H	>100	490
25	OH	H		>300
26	OCH_3	H		>1000
27	CN	H		>1000
28	H	F		32
2	H	Cl	0.56	7.0
29	H	Br	0.85	8.0
30	H	I	3.6	47
31	H	CF_3		300
32	H	CH_3	1.8	34
33	H	C_2H_5	>100	150
34	H	$CH{=}CH_2$	>100	263
35	H	*n*-C_3H_7	>100	>1000
36	H	*i*-C_3H_7	>100	>1000
37	H	*n*-C_4H_9	>100	>1000
38	H	$COCH_3$		>1000
39	H	OCH_3	>100	>1000
40	H	OCH_2Ph		>100
41	H	SCH_3		2130
42	H	NO_2		77
13	Cl	Cl	0.20	3.0
43	Br	Br	0.086	1.6
44	Br	CH_3	0.82	2.1
45	Br	C_2H_5	3.2	19
46	CH_3	Br	1.10	2.6
47	C_2H_5	Br	0.066	1.8
48	CH_3	CH_3	0.54	7.0
49	CH_3	I	1.2	9.0
50	I	CH_3	0.27	1.0
51	C_2H_5	Cl	0.26	1.0
52	Cl	C_2H_5	4.9	38
53	I	Cl	0.032	0.41
54	Cl	I	0.65	10
55	NO_2	NO_2	>100	>300
56	CF_3	CF_3	>100	>100

[a,b] See Table I.

(D-(+)-HA-966), D-cycloserine (6),[21] and 1-amino-1-carboxycyclobutane (7)[22] appear to be partial agonists at the glycine/NMDA site. Compounds having weaker potency include cycloleucine (8)[23] and 5-chloroindole-2-carboxylate (9).[24] Compound 7 is a more potent antagonist than the higher homologue 8, and further reduction in ring size to 1-amino-1-carboxycyclopropane[25] results in a potent glycine/NMDA agonist.

The kynurenic acid and quinoxaline derivatives have moderate potency and lack selectivity for the glycine/NMDA site. For example, compounds 1, 3, and 4 display equivalent or greater activity as competitive antagonists of the excitatory amino acid receptors sensitive to

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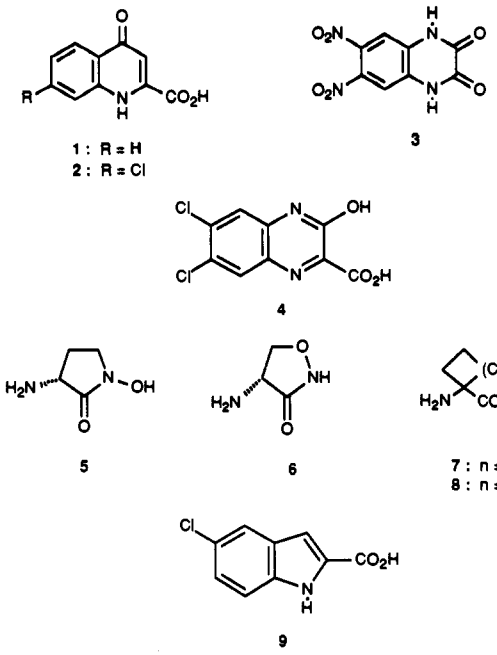


quisqualic acid or α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (QUIS or AMPA receptors) and kainic acid (KA receptors).[15,26] More potent and selective glycine/NMDA antagonists are required to investigate comparative neuroprotective and behavioral properties. We have synthesized an extensive series of compounds, based on kynurenic acid and quinoxaline-2,3-dione, in pursuit of enhanced antagonist potency and selectivity for excitatory amino acid receptor subtypes. Previously, we have shown that 7-chlorokynurenic acid (**2**) is a more potent and selective glycine/NMDA antagonist than kynurenic acid itself.[15] In this paper we report our results on the effects of aromatic ring substitution (compounds **10–65**, Tables I–V) and modifications of the heterocyclic moiety (compounds **66–81**, Tables VI–VIII). Specific 5,7-disubstituted kynurenic acid derivatives are shown to be the most potent and selective glycine/NMDA antagonists yet reported. Modification of the heterocyclic ring indicates that the 4-oxo and 1-amino groups are necessary for high activity. A simple model accounting for the binding of the known antagonists to the glycine/NMDA site is also presented.

Synthesis

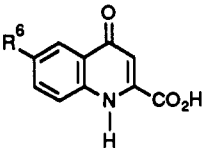
Kynurenic acid and quinoxaline derivatives were prepared by using established methods. Substituted kynurenic acid derivatives can be conveniently synthesized by the Conrad-Limpach[27,28] (Scheme I) and related procedures.[29] The required precursor anilines for 5- and 7-alkyl-substituted derivatives were prepared from 3-nitrobenzaldehyde and 3-nitroacetophenone by Wittig olefination followed by concomitant reduction of both nitro and olefinic groups.[30] 3-Halo-5-alkyl- and 3,5-dihaloanilines were derived from halogenation of the appropriate 4-substituted-2-nitroanilines, followed by removal of the amine and reduction of the nitro group.[30] Using 3-substituted anilines in the Conrad-Limpach procedure gives mixtures of 5- and 7-substituted kynurenic acid esters **83** and **84** following thermal ring closure of the enamine intermediate **82** (Scheme I). Use of 3,5-disubstituted anilines gave product mixtures where the major cyclized product invariably contained the bulkier substituent at the 7-position. The esters **83** and **84** were separated by either fractional crystallization or by column chromatography. The regioisomers were identified by ^{1}H NMR spectroscopy, using nuclear Overhauser effects where necessary. The identity of the halogenated derivatives **53** and **54** was determined by comparisons of ^{1}H NMR spectra with the corresponding

Table III. Substituent Parameters and Observed and Calculated Activities

				-log [1/Kb]	
no.	π	V-5	V-7	obsd	calcd[a]
1	0.00	2.52	2.52	3.812	3.812
15	0.14	5.80	2.52	4.387	4.117
10	0.71	12.00	2.52	4.432	4.722
16	0.86	15.12	2.52	4.699	4.847
17	1.12	19.64	2.52	5.119	4.953
18	0.88	21.33	2.52	4.456	4.733
19	0.56	13.67	2.52	4.678	4.641
20	1.02	23.90	2.52	5.260	4.708
22	1.55	34.13	2.52	3.398	4.193
24	2.13	44.36	2.52	3.310	3.084
28	0.14	2.52	5.80	4.495	4.304
2	0.71	2.52	12.00	5.154	4.872
29	0.86	2.52	15.12	5.097	4.785
30	1.12	2.52	19.64	4.328	4.354
31	0.88	2.52	21.33	3.523	3.864
32	0.56	2.52	13.67	4.469	4.694
33	1.02	2.52	23.90	3.824	3.354
34	0.82	2.52	19.31	3.580	4.209
41	0.61	2.52	24.47	2.672	2.926
42	−0.28	2.52	16.80	4.114	3.836
13	1.42	12.00	12.00	5.523	5.782
43	1.72	15.12	15.12	5.796	5.820
44	1.42	15.12	13.67	5.678	5.728
45	1.88	15.12	23.90	4.721	4.389
46	1.42	13.67	15.12	5.585	5.614
47	1.88	23.90	15.12	5.745	5.681
48	1.12	13.67	13.67	5.155	5.522
49	1.68	13.67	19.64	5.046	5.183
50	1.68	19.64	13.67	6.000	5.834
51	1.73	23.90	12.00	6.000	5.768
52	1.73	12.00	23.90	4.420	4.264
53	1.83	19.64	12.00	6.398	6.013
54	1.83	12.00	19.64	5.000	5.264

[a] From eq 1.

Table IV. 6-Substituted Kynurenic Acids



		Kb,[a] μM		
no.	R^6	NMDA	QUIS	KA
1	H	154	120	89
57	CH_3	IA@100	66	59
58	C_2H_5	IA@100	>100	>100
59	F	93	48	55
11	Cl	>100	30	19
60	Br	>100	28	30
61	I	100	56	37
62	CF_3	IA@100	>100	>100
63	SCH_3	>100	56	43
64	OCH_3	IA@100	87	32
65	NO_2	IA@100	52	49

[a] See Table I.

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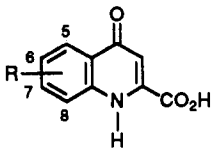
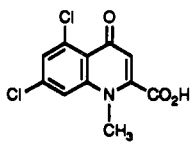
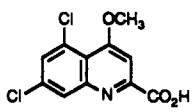
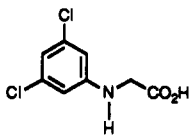
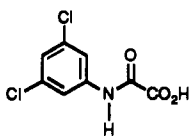
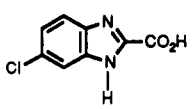
Table V. Binding Selectivity of Substituted Kynurenic Acids



no.	R	IC_{50},[a] μM Gly	Glu	AMPA	KA
1	H	41	184	101	>1000
11	6-Cl	>100	>300	41	>300
2	7-Cl	0.56	169	153	>1000
13	5,7-Cl_2	0.20	>100	75	>300
53	5-I, 7-Cl	0.032	>100	>100	>100

[a] Inhibition of binding of ^3H-labeled ligands labeling excitatory amino acid receptors (refs 15 and 19). Gly, glycine; Glu, L-glutamate; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate; KA, kainic acid.

Table VI. Heterocyclic Ring Modified Derivatives

no.	structure	[^{3}H]Gly IC_{50},[a] μM	Kb,[b] μM NMDA	QUIS	KA
66		>100	IA@100	IA@100	IA@100
67		~100	IA@100	IA@100	IA@100
68		49	314	IA@100	
69		6.8	41	IA@100	
70		5.6	17	101	

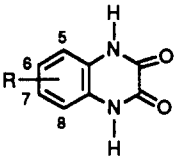
[a,b] See Table I.

monosubstituted derivatives and was confirmed by hydrogenolytic deiodination of **53** to 7-chlorokynurenic acid (**2**). The *N*-methyl derivative **66** was synthesized from the appropriate *N*-methylaniline and the 4-*O*-methyl isomer **67** was obtained via methanolysis of the 4-chloro precursor.[27] The *N*-methylquinoxaline-2,3-dione **78** was obtained by oxidation[31] of the corresponding 3-deoxy precursor. Methylation of **76** gave the bis-*N*-methylated compound **80** and the O-methylated derivatives **79** and **81** were obtained from methanolysis[32,33] of the corresponding dichloride. Physical properties of new compounds are given in Table IX.

Biology

Compounds were evaluated in in vitro assays predictive for activity at NMDA, QUIS, and KA receptors. Details

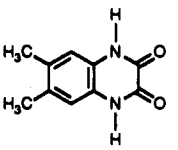
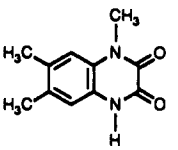
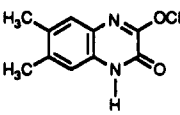
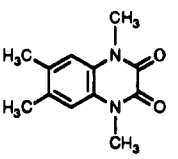
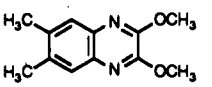
Table VII. Substituted Quinoxaline-2,3-diones



no.	R	[^{3}H]Gly IC_{50},[a] μM	Kb,[b] μM NMDA	QUIS	KA
71	H	38.6	52	>100	>100
72	5-Cl	56.3	47	204	>100
73	7-Cl	8.4	13	19	26
74	5,7-Cl_2	3.4	7.0	27	22
75	6,7-Cl_2	3.1	6.0	13	9
76	6,7-$(CH_3)_2$	2.3	6.6	16	27
77	6-NO_2	8.3	5.6	5.7	4.6
3	6,7-$(NO_2)_2$	4.5	5.3	0.59	1.29

[a,b] See Table I.

Table VIII. Methylated Derivatives of 6,7-Dimethylquinoxaline-2,3-dione (**76**)

no.	structure	[^{3}H]Gly IC_{50},[a] μM	Kb,[b] μM NMDA	QUIS	KA
76		2.3	6.6	16	27
78		6.6	130	43	47
79		>100	IA@30	IA@30	86
80		>100			
81		>100			

[a,b] See Table I.

of the methodologies used have been published.[15,19] Affinities for neurotransmitter recognition sites on excitatory amino acid receptors were determined by using radioligand binding to rat cortical membranes. The concentrations of test compounds required to inhibit 50% of the specific binding (IC_{50}'s) of [^{3}H]glycine, [^{3}H]glutamate, [^{3}H]AMPA, and [^{3}H]kainate were indicative of affinities at glycine/NMDA, NMDA, QUIS, and KA receptor sites, respectively. Functional antagonist potency was assessed by determination of the apparent dissociation constants (Kb's) of test compounds for antagonism of the depolarizations induced by *N*-methyl-D-aspartic acid, quisqualic acid, and kainic acid in a rat cortical slice preparation. In this functional test, all of the compounds having quisqualate antagonist activity also blocked kainate responses with a similar potency. However none of these QUIS/KA antagonists was able to reduce [^{3}H]kainate binding, suggesting that the high affinity [^{3}H]kainate site is not the

(31) St. Clair, R. L.; Thibault, T. D. U.S. Pat. 3,992,378, 1976.
(32) Cheeseman, G. W. H. *J. Chem. Soc.* **1955**, 1804.
(33) Patton, R.; Schulz, H. P. *J. Am. Chem. Soc.* **1951**, *73*, 5899.

Table IX. Physical Data of New Compounds

no.	method[a]	mp,[b] °C	formula	analyses	^{1}H NMR δ (ppm)[c] 3-H	5-H	6-H	7-H	8-H
14		290	$C_{10}H_4Cl_3NO_3$	C,H,N	6.69				7.66[d]
15	A	285–286	$C_{10}H_6FNO_3$	e	6.86		7.52	7.04	7.68[d]
16	B	293	$C_{10}H_6BrNO_3$	C,H,N	6.92		7.62	7.42	7.80[d]
17	B	286–287	$C_{10}H_6INO_3 \cdot 0.5H_2O$	C,H,N	6.75		7.87	7.08	7.66[d]
18	C	265–267	$C_{11}H_6F_6NO_3 \cdot H_2O$	C,H,N	6.65		7.78	7.78	8.26
20	D	272–273	$C_{12}H_{11}NO_3$	C,H,N	6.65		7.18	7.54	7.68[d]
21	E	265–266	$C_{12}H_9NO_3 \cdot 0.1H_2O$	C,H,N	6.56		7.37	7.62	7.91
22	E	268	$C_{13}H_{13}NO_3$	C,H,N	6.53		7.03	7.51	7.80
23	E	263–265	$C_{13}H_{13}NO_3 \cdot 0.1H_2O$	C,H,N	6.53		7.26	7.56	7.79
24	E	269–270	$C_{14}H_{15}NO_3 \cdot 0.2H_2O$	C,H,N	6.53		7.03	7.50	7.78
25	F	298–301	$C_{10}H_7NO_4 \cdot 0.2H_2O$	C,H,N	6.62		6.60	7.54	7.34
26	C	231–233	$C_{11}H_9NO_4 \cdot H_2O$	C,H,N	6.45		6.77	7.53	7.44
27	G	278–279	$C_{11}H_6N_2O_3 \cdot 0.3H_2O$	C,H,N	6.96		7.83	7.64	8.05[d]
28	A	289–290	$C_{10}H_6FNO_3 \cdot 0.3H_2O$	C,H,N	6.61	8.12	7.23		7.69
33	D	271	$C_{12}H_{11}NO_3$	C,H,N	6.66	8.11	7.36		7.66[d]
34	E	281	$C_{12}H_9NO_3 \cdot 0.6H_2O$	C,H,N	6.61	8.05	7.54		7.94
35	E	268–270	$C_{13}H_{13}NO_3 \cdot 0.6H_2O$	C,H,N	6.59	7.98	7.20		7.74
36	E	253–254	$C_{13}H_{13}NO_3$	C,H,N	6.58	8.00	7.27		7.82
37	E	247–250	$C_{14}H_{15}NO_3 \cdot 0.5H_2O$	C,H,N	6.60	7.98	7.20		7.75
40	H	278–279	$C_{17}H_{13}NO_4$	C,H,N	6.83	8.10	7.12		7.35[d]
41	H	294–295	$C_{11}H_9NO_3S$	C,H,N	6.89	8.08	7.31		7.61[d]
44	J	298	$C_{11}H_6BrNO_3 \cdot 0.65H_2O$	C,H,N	6.88		7.45		7.54[d]
45	E	276	$C_{12}H_{10}BrNO_3 \cdot 0.9H_2O$	C,H,N	6.54		7.42		7.79
46	J	299	$C_{11}H_8BrNO_3$	C,H,N	6.85		7.22		7.83[d]
47	E	278	$C_{12}H_{10}BrNO_3 \cdot 0.3H_2O$	C,N,N	6.55		7.22		8.02
48		285–287	$C_{12}H_{11}NO_3$	C,H,N	6.49		6.87		7.55
49	K	293–295	$C_{11}H_8INO_3$	C,H,N	6.50		7.37		8.20
50	K	305–306	$C_{11}H_8INO_3 \cdot 1.1H_2O$	C,H,N	6.55		7.75		7.75
51	E	282–283	$C_{12}H_{10}ClNO_3 \cdot H_2O$	C,H,N	6.52		7.20		7.75
53	L	298	$C_{10}H_5ClINO_3 \cdot 1.5H_2O$	C,H,N	6.58		7.88		8.09
54	L	275	$C_{10}H_5ClINO_3$	C,H,N	6.55		7.62		8.34
55		285	$C_{10}H_5N_3O_7 \cdot 0.35H_2O$	C,H;N[f]	6.76		9.07		8.43
56		258	$C_{12}H_5F_6NO_3 \cdot 0.9H_2O$	C,H,N	6.30		8.64		8.03
62		287–289	$C_{11}H_6F_3NO_3 \cdot 0.75H_2O$	C,H,N	6.72	8.33		8.01	8.14
63		256–257	$C_{11}H_9NO_3S \cdot 0.5H_2O$	C,H,N	6.65	7.83		7.62	7.90
66		210	$C_{11}H_7Cl_2NO_3$	C,H,N	6.34		7.53		7.84
67		205–206	$C_{11}H_7Cl_2NO_3$	C,H,N	7.59		7.85		8.11
68		148–149	$C_8H_7Cl_2NO_2$	C,H,N	g				
69		206–207	$C_8H_5Cl_2NO_3$	C,H,N	h				
72		315–318	$C_8H_5ClN_2O_2$	C,H,N			7.09	7.20	7.09
74		337–340	$C_8H_4Cl_2N_2O_2$	C,H,N			7.35		7.09
78		338–346	$C_{11}H_{12}N_2O_2$	C,H,N		7.15			6.92
79		263–273	$C_{11}H_{12}N_2O_2$	C,H,N		7.32			7.00
80		293–295	$C_{12}H_{14}N_2O_2$	C,H,N		7.21			7.21
81		142–144	$C_{12}H_{14}N_2O_2$	C,H,N		7.53			7.53

[a] Method used to separate 5- and 7-substituted kynurenic acid regioisomers: A, chromatography of methyl esters on silica gel, eluent 15–30% HOAc/toluene; B, ethyl esters recrystallized from pyridine and then aqueous EtOH; C, methyl esters recrystallized from pyridine; D, chromatography of methyl esters on silica gel, eluent 2.5% MeOH/CH_2Cl_2; E, chromatography of methyl esters on silica gel, eluent 10–60% EtOAc/hexane; F, methyl esters recrystallized from MeOH; G, methyl esters recrystallized from AcOH/EtOH and then pyridine; H, only one regioisomer isolated; J, chromatography of methyl esters on silica gel, eluent 6% MeOH/CH_2Cl_2; K, reverse phase chromatography of the acids using Deltapak RP18 column on Waters Prep 3000, gradient elution, 95:5 to 5:95 0.1% CF_3CO_2H/H_2O:0.1% CF_3CO_2H/CH_3CN; L, reverse-phase chromatography of the acids (see the Experimental Section). [b] Kynurenic acids generally melt with decomposition. [c] In DMSO-d_6, unless stated. [d] In NaOD/D_2O. [e] MS: m/e 207 (M^+). [f] N: calcd, 14.26; found, 14.72. [g] 3.85 (2 H, d, CH_2), 6.58 (2 H, d, Ph-2,6-H), and 6.64 (1 H, t, Ph-4-H). [h] 7.37 (1 H, t, Ph-4-H), 7.89 (2 H, d, Ph-2,6-H), and 11.02 (1 H, bs, NH).

receptor through which kainate produces depolarizations of cortical tissue. The correlation between Kb's for QUIS/KA antagonism and affinity for [^{3}H]AMPA binding indicates that the responses to both of these agonists may be mediated by the same "quisqualate-type" receptor.

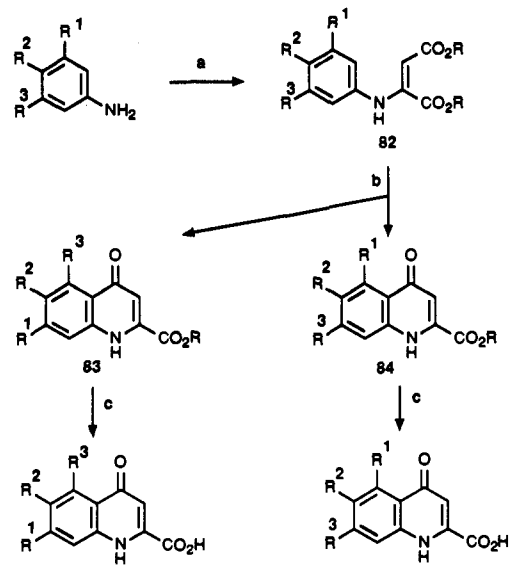
The IC_{50}'s from the radioligand-binding assays were obtained from at least two experiments, and the values given are the means of two to four determinations; the maximum variance (geometric mean) was 60%. The Kb values from the cortical slice assay are means from at least three experiments; the maximum variance (geometric mean) was 15%. Generally, IC_{50}'s of compounds in the [^{3}H]glycine binding assay are around 10-fold lower than apparent Kb's for *N*-methyl-D-aspartate antagonism in the cortical slice. The lower activity in the functional assay is probably a consequence of competition with the supersaturating levels of endogenous glycine present in the cortical slice preparation. The potencies of selected compounds as antagonists of glycine-induced NMDA currents in isolated cultured neurones, where glycine concentrations can be adjusted, were fully consistent with their affinities for the [^{3}H]glycine site.[15,34]

Substitution of the Benzene Ring

Our initial finding that glycine/NMDA antagonist potency and selectivity was enhanced by 7-chloro substitution (**2**)[15] led us to investigate the effects of chloro substitution at the 5-, 6-, and 8-positions (Table I). Kynurenic acid itself (**1**) is a nonselective and only weakly active excitatory amino acid antagonist, which has been shown to act with similar potencies at both the glycine and NMDA sites on the NMDA receptor as well as at QUIS/KA receptors.[15]

(34) Kemp, J. A.; Priestley, T. Unpublished results.

Scheme I[a]



[a] (a) RO_2C–CC–CO_2R or RO_2C–$COCH_2$–CO_2R. (b) Ph_2O/250 °C or PPA. (c) NaOH.

Chloro substitution at both the 5-position (10) and 7-position (2) results in enhanced potency at the glycine/NMDA site, but does not change QUIS/KA antagonism. In contrast, 6-chloro substitution (11) selectively enhances activity at both non-NMDA receptors, and the 8-chloro derivative (12) is essentially inactive at all excitatory amino acid receptors. 8-Hydroxykynurenic acid is also known to lack antagonist activity.[35] The activities of the 5,7-dichloro (13) and 5,6,7-trichloro (14) derivatives show that both potency and selectivity appear to be additive. Thus 13 is a more potent and selective glycine/NMDA antagonist than either of the monosubstituted compounds 2 and 10. Addition of a 6-chloro substituent (14) reduces glycine/NMDA activity, but enhances non-NMDA activity, resulting in a potent broad-spectrum antagonist. The results show that the analogues in Table I are able to distinguish between NMDA and non-NMDA receptors as a result of only minor changes in substitution.

The trends apparent in Table I were further explored by individual modifications of substituents at the 5-, 6-, and 7-positions. A series of 5,7-substituted analogues were made to identify optimal glycine/NMDA antagonist potency and selectivity. Each substituent position was independently optimized and a series of 5,7-disubstituted analogues were prepared (Table II). At the 5-position, halogen substitution results in increasing activity with increasing size, with the iodo analogue 17 having 65-fold improved affinity for the [^{3}H]glycine site relative to kynurenic acid (1). The bulkier trifluoromethyl derivative (18) however does not improve activity to the same extent. Alkyl substitution shows a similar trend, with activity increasing up to ethyl (20). However, further increases in size result in sharp decreases in activity, with the *n*-propyl (22) and *n*-butyl (24) compounds having weaker activity than kynurenic acid. The greatly reduced activity of the vinyl derivative (21) relative to the ethyl compound (20) suggests the existence of a very strictly size limited binding site. Introduction of small hydrophilic groups such as hydroxy (25), methoxy (26), and cyano (27) causes complete loss of activity, indicating that this binding pocket on the glycine/NMDA receptor is essentially hydrophobic.

The overall structure–activity trends at the 7-position are similar to those observed at the 5-position, but steric tolerance is even further limited. Thus the chloro (2) and bromo (29) compounds have greater potency than the fluoro (28) or iodo (30) derivatives, and the methyl analogue (32) is more potent than the ethyl homologue (33). In contrast to the 5-position, 7-chloro and -bromo substituents are superior to methyl, suggesting the possibility that electron withdrawal or a 7-electronegative group may weakly enhance binding. Introduction of larger or hydrophilic groups (36–41) abolishes activity, but the nitro derivative (42) has twice the potency of kynurenic acid.

Combinations of potency-enhancing 5- and 7-substituents have an additive effect on activity, as illustrated by the chloro-, bromo-, iodo-, methyl-, and ethyl-substituted analogues (13 and 43–54). Thus improved activity was seen with ethyl or iodo groups at the 5-position together with chloro or bromo groups at the 7-position, for example 47, 51, and 53. The most active compound proved to be 5-iodo-7-chlorokynurenic acid (53), having an IC_{50} of 32 nM, indicating a 1000-fold improved affinity at the glycine/NMDA site relative to kynurenic acid itself.

The structure–activity studies suggest that activity is enhanced selectively at the glycine/NMDA site by specific hydrophobic binding of the 5- and 7-substituents. The apparent additivity of substituent effects was confirmed by construction of quantitative structure–activity relationships (QSARs). Values for substituent hydrophobicity[36] and volume[37] were taken from the extensive compilations available in the literature. Initial QSARs of the individual 5- and 7-substituted series confirmed the apparent trend of optimal size and hydrophobicity, and the NMDA antagonist activities (apparent Kb, Table II) of all compounds in the cortical slice assay were adequately described by the following equation:

$$-\log [1/\mathrm{Kb}] = 0.675\pi + 0.0890\mathrm{V\text{-}5} - 0.00300(\mathrm{V\text{-}5})^2 + 0.201\mathrm{V\text{-}7} - 0.00965(\mathrm{V\text{-}7})^2 + 3.16 \quad (1)$$

$$n = 33,\ r = 0.937,\ s = 0.338,\ F = 38.57\ (p < 0.0001)$$

$$t_\pi = 2.74\ (p = 0.0107),\ t_{\mathrm{V\text{-}5}} = 3.44\ (p = 0.001\,89),\ t_{(\mathrm{V\text{-}5})2} = 6.77\ (p < 0.0001),\ t_{\mathrm{V\text{-}7}} = 5.39\ (p < 0.0001),\ t_{(\mathrm{V\text{-}7})2} = 7.60\ (p < 0.001)$$

In eq 1, π is the summed hydrophobicity constant contant for the 5,7-substituents, derived from octanol/water partition coefficients[36] of substituted benzene derivatives; V-5 and V-7 are the van der Waals volumes[37] in cubic angstroms of the 5- and 7-substituents, respectively; *n* is the number of compounds in the analysis; *r* is the correlation coefficient; *s* is the standard deviation; *F* is the variance ratio; and *t* is the ratio of the coefficient to its standard deviation and is significant at the level given (*p*). The substituent constants used, together with observed and predicted potencies, are given in Table III.

Equation 1 shows that NMDA antagonist activity is enhanced by hydrophobic and optimally sized 5,7-substituents. The differing coefficients in the volume terms V-5 and V-7 reflect the greater tolerance to bulky substituents at the 5-position. Equation 1 successfully predicts the activity of the most (53) and least (41) active analogues. The weak activity of other compounds whose apparent Kb's could not be determined accurately (usually because of poor solubility at the high concentrations required) are entirely consistent with eq 1, with the exception of the

(35) Robinson, M. E.; Schulte, M. K.; Freund, R. K.; Johnson, R. L.; Koerner, J. F. *Brain Res.* **1985**, *361*, 19.

(36) Hansch, C.; Leo, A. *Substituent Constants for Correlation Analysis in Chemistry and Biology*; Wiley-Interscience: New York, 1979.

(37) Bondi, A. *J. Phys. Chem.* **1964**, *68*, 441.

5-vinyl analogue (21). On the basis of hydrophobic and volume requirements alone, compound 21 should be as active as the iodo (17) and ethyl (20) derivatives. The reduced activity of 21 may be a consequence of a specific directional steric interaction. However, use of substituent dimensions, as well as specific electronic parameters, in the analysis did not yield any significant improvement to eq 1.

A group of 6-substituted derivatives were evaluated to define preliminary structure–activity relationships for activity at non-NMDA receptors. The results (Table IV) show that, with the exception of the fluoro derivative (59), QUIS/KA antagonist selectivity is retained in the compounds tested. However, unlike the 5,7-substituted derivatives, there is no obvious substitution-dependent trend for non-NMDA antagonist activity. The ethyl (58) and trifluoromethyl (62) analogues are the least potent. For each compound, essentially equivalent potencies for inhibition of QUIS and KA responses were found.

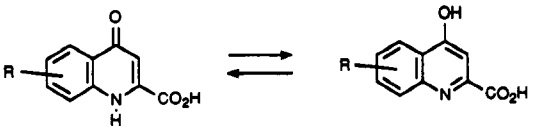
The effects of 5,6,7-substitution on selective binding affinity for each of the excitatory amino acid receptor subtypes is summarized for the key compounds in Table V. The 5,7-substituted compounds (2, 13, and 53) have markedly enhanced selectivity for displacement of [^{3}H]-glycine binding relative to kynurenic acid (1). Comparisons of the relative affinities of 13 and 53 for the [^{3}H]glutamate and [^{3}H]glycine binding sites strongly suggests that the NMDA antagonist activities of these compounds is a consequence of selective glycine site blockade. Other evidence in support of this conclusion includes the following: (a) NMDA antagonism on cortical slices cannot be overcome by increasing the NMDA concentration (i.e. it is noncompetitive), but can be reversed by addition of the glycine site agonists, glycine or D-serine;[15] (b) there is a clear correlation between Kb values for NMDA antagonism and IC_{50}'s for inhibition of [^{3}H]glycine binding (Table II). In contrast, the selective non-NMDA activity of the 6-chloro analogue (11) is consistent with reduced affinity for the [^{3}H]glycine site and improved affinity at the QUIS/AMPA receptor.

These results show that aromatic substitution of the broad-spectrum excitatory amino acid antagonist kynurenic acid (1) results in 5,7-substituted analogues having selectivity for the glycine/NMDA site (Table II) whereas 6-substitution (Table IV) results in selective non-NMDA antagonism. The structure–activity relationships suggest that optimal size and hydrophobicity can enhance activity of the 5,7-substituted derivatives for the glycine/NMDA site (eq 1), but there are less strict requirements for the activity of 6-substituted analogues at non-NMDA receptors. Thus the transmitter recognition site of the QUIS/AMPA receptor may share some structural resemblance to the glycine/NMDA site.[26] The kynurenic acid derivatives appear to distinguish between these receptor sites as a consequence of selective hydrophobic binding interactions of the differently substituted benzene rings (Table V).

Modification of the Heterocyclic Ring

Kynurenic acid derivatives can exist as both 4-keto (I) and 4-hydroxy (II) tautomers (Scheme II). Previously, it has been shown that removal of the 4-oxo substituent from kynurenic acid results in loss of antagonist activity in a rat hippocampal slice test.[35] However, this loss of activity cannot be ascribed to the absence of the 4-substituent alone, since the resulting 2-carboxyquinoline cannot tautomerize and lacks a proton on the nitrogen atom. We have tested several heterocyclic ring modified analogues to help identify the individual roles of the 1-amino and 4-oxo substituents in recognition by the glycine/NMDA receptor (Table VI).

Scheme II



I II

Comparisons of the ^{13}C NMR spectra of 5,7-dichlorokynurenic acid (13) with the N-methyl and 4-methoxy derivatives (66 and 67, respectively) confirm that the 4-keto tautomer (I) is preferred in polar solvents (see the Experimental Section). The actual tautomer that binds to the glycine site cannot be easily ascertained, since both methylated derivatives 66 and 67 lack antagonist activity (Table VI). This may be explained by steric effects due to the methyl groups, or by loss of an essential receptor hydrogen bonding interaction requiring the proton on either the hydroxyl of II or the nitrogen of I.

Deletion of the heterocyclic ring results in retention of weak antagonist activity and glycine site affinity, as shown by the arylglycine 68 and the oxanilide 69. Since both 68 and 69 must have a proton attached to nitrogen, they can be regarded as mimics of the keto tautomer (I). Furthermore, the increased activity of 69 relative to 68 suggests that the carbonyl group in the oxanilide 69 may mimic the 4-keto group in the tautomer I. These results led us to examine 2-carboxybenzimidazoles (e.g. 70, Table VI) as cyclic analogues of the oxanilide 69. Compound 70 (IC_{50} = 5.6 μM) proved to have improved affinity for the [^{3}H]glycine binding site relative to the corresponding indole (9,[24] IC_{50} in our hands >100 μM). Thus the cyclic 3-imino group in 70 may mimic both the amide carbonyl in 69 and the 4-keto group in the kynurenic acids, resulting in enhanced binding to the glycine site. Notably, the 2-carboxybenzimidazole 70 also possesses appreciable non-NMDA antagonist activity. However these results indicate that in the kynurenic acid derivatives the keto tautomer I may be recognized by the glycine/NMDA site, and that both the 4-keto and 1-NH groups are necessary for binding.

The quinoxaline-2,3-diones (e.g. 3) are of structural interest as glycine/NMDA antagonists, since they lack the carboxyl group that is obligatory[35] in the kynurenic acids. However, the compounds reported to date[16,17] are not selective for the glycine site and 3[38] and other derivatives[39] are selective antagonists at non-NMDA receptors. We have tested selected quinoxaline-2,3-dione derivatives to establish the extent of comparability of aromatic substitution with the kynurenic acids (Table VII).

Addition of chloro groups at the 7-position leads to an enhancement of activity of the quinoxalinediones 73 and 74 at the glycine/NMDA site, but the improvement is less than was found in the correponding kynurenic acid derivatives 2 and 13 (Table I) and also differs in that it is accompanied by an equivalent increase in QUIS/KA antagonist activity. In addition, 5-chloro substitution has little effect as shown by the similar activities of 71 and 72 and also 73 and 74. Thus the 5,7-substituents in the kynurenic acids and the quinoxalines are recognized differently by the QUIS receptor. Further, aromatic ring substitution by chloro (75), nitro (77 and 3), and methyl

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(76) at the 6- and 7-positions does not change glycine/NMDA antagonist activity in the quinoxaline series, in complete contrast to the effects of these substitutions in the kynurenic acids. The 6-nitro derivative 77 is a potent broad-spectrum excitatory amino acid antagonist, and addition of the 7-nitro group clearly enhances the non-NMDA actions of the dinitro derivative 3.[38] The quinoxalinediones were found to have comparable IC_{50} and Kb values (Table VII), in contrast with the kynurenic acids, where IC_{50}'s are generally around 10-fold lower than Kb's (Table II). The reasons for this difference are unclear at present.

Since the quinoxaline-2,3-dione structure can exist in three possible tautomeric forms, several O- and N-methylated derivatives of the symmetrical 6,7-disubstituted analogue 76 were tested (Table VIII). N-Monomethylation (78) results in reduced antagonist activity at the glycine/NMDA site, but significantly QUIS/KA antagonism is only slightly reduced. O-Monomethylation (79) however reduces activity at all receptors. Dimethylation at either nitrogen (80) or oxygen (81) abolishes activity. Although comparisons of the ^{13}C NMR spectra of the methylated compounds (78–81) with the unsubstituted analogue (76) confirmed that these quinoxalines exist predominantly as the 2,3-dione tautomers in solution (see the Experimental Section), the identity of the tautomer responsible for glycine/NMDA receptor antagonism cannot be readily determined from these results. However, it is apparent that both protons attached to nitrogen or oxygen are important for binding to the glycine/NMDA site, but only one is required for recognition by non-NMDA receptors.

The overall structure–activity relationships for excitatory amino acid antagonism in the kynurenic acid and quinoxalinedione series are distinctly different. Although both classes provide compounds that act competitively at glycine/NMDA and QUIS/KA receptors, they cannot be considered to be completely congeneric series. However comparisons of the structures of the ligands having antagonist activity at the glycine/NMDA site has provided a model that can help to explain the different structure–activity trends.

Glycine/NMDA Antagonist Binding Model

On the basis of the above structure–activity relationships, we suggest that recognition of the kynurenic acid antagonists by the glycine/NMDA receptor requires (a) size-limited hydrophobic binding of the benzene ring, (b) a Coulombic or hydrogen-bonding interaction of the 2-carboxylate, (c) hydrogen-bond donation from the 1-NH group, and (d) hydrogen-bond acceptance by the 4-keto group. The proposed model is shown for compound 13 in Figure 1a. Interestingly, the structural requirements for glycine/NMDA agonist binding[40-42] suggest hydrogen-bond acceptance may be involved in the binding of the side chains of D-serine and D-(fluoromethyl)alanine.[42] However agonist binding differs from antagonist binding in that only small D-amino acid substituents are allowed and substitution on nitrogen reduces affinity, suggesting that a zwitterionic amino acid is essential for receptor activation. Although a glycine part-structure is clearly present in the kynurenic acids, the nitrogen atom differs from glycine

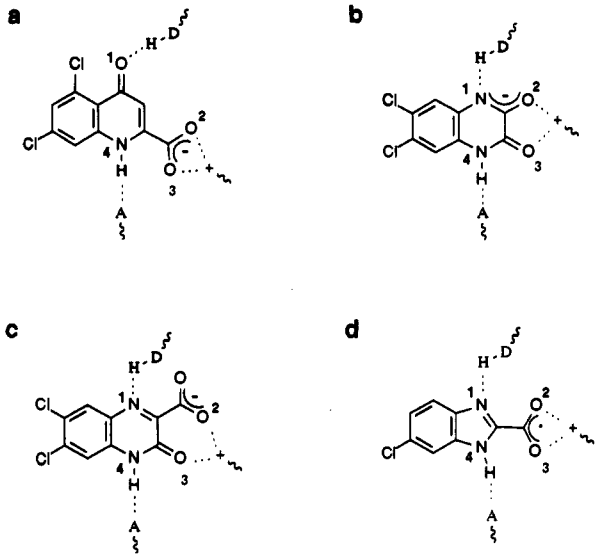

Figure 1. Proposed hydrogen-bonding and Coulombic receptor interactions of glycine/NMDA antagonists: (a) kynurenic acids 13; (b) quinoxaline-2,3-diones 75; (c) quinoxalic acids 4; (d) 2-carboxybenzimidazoles 70. D-H and A represent a receptor hydrogen bond donor and acceptor, respectively; + represents a positively charged receptor group.

itself in that it is insufficiently basic to become protonated at physiological pH. While this glycine moiety may bind to the receptor groups that recognize the agonist, the hydrophobic benzene ring, combined with the nonbasic nitrogen atom, may be important for interaction with an antagonist-preferring receptor conformation. The strict size tolerance observed at the 5-position (Table II) may in part be due to steric inhibition of binding of the adjacent 4-keto group.

The model can be used to propose binding interactions of quinoxaline-2,3-diones (Figure 1b), quinoxalic acids (Figure 1c), and 2-carboxybenzimidazoles (Figure 1d). In each case it is suggested that the cyclic imino group at the 4-position can act as the hydrogen-bond acceptor. Although other modes of binding are possible for the quinoxaline derivatives, the requirement for an anionic species implies that the 3-oxo anion of the quinoxaline-2,3-dione must bind to the active site (Figure 1b). Similarly, the quinoxalic acid (Figure 1c) is proposed to bind with the anionic carboxyl group located at the equivalent 3-position. Alternatively, protonation of the cyclic imino nitrogen of the quinoxalic acid at the receptor would allow a similar mode of binding to the kynurenic acids (not shown) where this nitrogen (labeled 1 in Figure 1c) would mimic the 1-amino group in kynurenic acids (labeled 4 in Figure 1a).

The structural relationships between key receptor binding groups in these molecules were further examined by molecular modeling. The molecules in Figure 1 have essentially flat structures and consequently a convincing 3-dimensional pharmacophore cannot be readily established. However the rigidity of the structures allows 2-dimensional molecular superimposition of the key pharmacophoric groups, as shown in Figure 2. Each of the molecules 4, 70, and 75 were fitted to the kynurenic acid 13 by least-squares superimpositions of the groups 1–4 proposed to be necessary for the key hydrogen-bonding and charge interactions (Figure 1). By these criteria, the benzene rings of both quinoxalines and the benzimidazole are well fitted, but are located in a region distinct from the space occupied by the aromatic ring of the kynurenic acid (Figure 2). This could explain the observed differences in both glycine/NMDA structure–activity trends and

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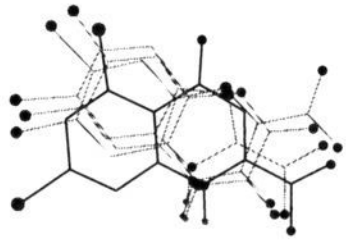

Figure 2. Superimposition of the molecules **13** (bold bonds) and **4**, **70**, and **75** (light bonds). The atoms labeled 1–4 in Figure 1 were matched by a least-squares-fitting procedure. The modeling was done using MOLEDIT/OPTIMOL (MSDRL's in-house software, written by the Molecular Systems Group, Rahway, NJ) and CHEMX (Chemical Design Ltd, Oxford, U.K.).

in selectivity between the kynurenic acids and the other ligands. An additional feature that may be significant in this respect is the different location of the hydrogen bond accepting 4-oxo substituent in the kynurenic acids relative to the corresponding nitrogen atoms in the other antagonists.

Conclusions

These studies have shown that specific 5,7-disubstituted kynurenic acids are highly potent and selective antagonists at the glycine modulatory/coagonist site on the NMDA receptor. Substitution at the 6-position in kynurenic acid however results in antagonist activity at non-NMDA receptors. Modifications of the heterocyclic ring suggest that the 4-keto tautomer may be required for binding to the glycine/NMDA site. A simple binding model can account for glycine/NMDA receptor recognition of the kynurenic acids, and the structurally related but less selective quinoxaline-2,3-diones, quinoxalic acids, and 2-carboxybenzimidazoles. The results indicate that the glycine/NMDA site may share some structural resemblance to the transmitter recognition site on the QUIS/AMPA receptor, and provide a basis for the design of further antagonists possessing high potency and selectivity.

Experimental Section

General directions have appeared previously.[10]

7-Chloro-5-iodo-4-oxo-1,4-dihydroquinoline-2-carboxylic Acid (53). To a solution of 4-chloro-2-nitroaniline (100 g, 0.58 mol) in glacial HOAc (800 mL) was added iodine monochloride (98 g, 0.60 mol). The mixture was heated at 120 °C for 3 h, cooled, and then poured into excess iced water. The precipitate that formed was collected, washed with 10% sodium sulfite solution, and then recrystallized from MeOH to give 4-chloro-2-iodo-6-nitroaniline (60 g, 35%), mp 135 °C. To a stirred solution of this aniline (43 g, 0.144 mol) in EtOH (700 mL) was added, dropwise, concentrated sulfuric acid (27.09 mL). The mixture was heated to reflux, and solid sodium nitrite (24.8 g, 0.42 mol) was added in small portions over a period of 30 min. The mixture was refluxed for a further 1 h, cooled, and poured into excess iced water. The mixture was extracted with EtOAc, and evaporated to yield crude 5-chloro-3-iodonitrobenzene (40 g), mp 65 °C. A stirred suspension containing this nitrobenzene (3.32 g, 0.0117 mol) and iron powder (4.3 g, 0.077 g atom) in HOAc (10 mL) and EtOH (35 mL) was refluxed for 6 h. The mixture was cooled, filtered, and added to excess water. The aqueous mixture was extracted with EtOAc and the organic extract dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography on silica gel, eluting with 5% EtOAc in hexane, to give 3-chloro-5-iodoaniline (2.4 g, 81%): bp 98 °C (0.06 mm); ^1H NMR (360 MHz, DMSO-d_6) δ 8.28 (1 H, d, 2-H), 8.35 (1 H, d, 6-H), and 8.46 (1 H, d, 4-H).

A solution of 3-chloro-5-iodoaniline (1.4 g, 0.0055 mol) and diethyl acetylenedicarboxylate (0.99 g, 0.0058 mol) in MeOH (10 mL) was refluxed for 10 h, the solvent removed by evaporation and the residue added to stirred diphenyl ether (20 mL), which had been preheated to 250 °C. After 15 min, the mixture was cooled and the resulting precipitate collected and washed with hexane to give a mixture (1.83 g, 88%) of ethyl 5-chloro-7-iodo-4-oxo-1,4-dihydroquinoline-2-carboxylate [^1H NMR (360 MHz, DMSO-d_6) δ 1.37 (3 H, t, CH_3), 4.40 (2 H, q, CH_2), 6.59 (1 H, s, 3-H), 7.61 (1 H, d, 6-H), 8.32 (1 H, d, 8-H), and 11.90 (1 H, bs, NH)] and ethyl 7-chloro-5-iodo-4-oxo-1,4-dihydroquinoline-2-carboxylate [^1H NMR (360 MHz, DMSO-d_6) δ 1.37 (3 H, t, CH_3), 4.40 (2 H, q, CH_2), 6.63 (1 H, s, 3-H), 7.90 (1 H, d, 6-H), 8.04 (1 H, d, 8-H), and 11.90 (1 H, bs, NH)]. The mixture was saponified with sodium hydroxide[27] and a portion (0.15 g) of the resultant mixture of **53** and **54** separated by reverse-phase chromatography on a Deltapak RP18 column using a Waters Prep 3000 instrument, eluting with $H_2O/CH_3N/DMF/CF_3CO_2H$ (55:22.5:22.5:0.1), to give pure **53** (8 mg, Table IX). Hydrogenolysis of **53** over Pd/C gave **2**, which was identical with an authentic sample.[27]

The other new 5,7-substituted kynurenic acids (Table II) were prepared[30] as described above. In most cases the mixtures of regioisomers were separated as the ester derivatives by either crystallization or chromatography (see Table IX). The kynurenic acids were recrystallized from hot aqueous ethanolic sodium hydroxide on addition of 2 N hydrochloric acid to pH 2.

5,7-Dichloro-1-methyl-4-oxo-1,4-dihydroquinoline-2-carboxylate (66). A solution of acetyl chloride (2.86 g, 0.0364 mol) in tetrahydrofuran (THF, 5 mL) was added dropwise to a stirred cooled (0 °C) solution of 3,5-dichloroaniline (5 g, 0.0309 mol) and triethylamine (3.75 g, 0.037 mol) in THF (5 mL). The solution was warmed to room temperature, then water was added, and the mixture was extracted with EtOAc. The extracts were washed with water, 2 N HCl, saturated $NaHCO_3$, and brine, dried over $MgSO_4$, and evaporated. The resulting crude *N*-acetate was dissolved in THF and the solution cooled with stirring to −78 °C. A solution of *n*-butyllithium in hexane (33.5 mmol) was added, the solution warmed to −10 °C and after 30 min recooled to −78 °C, and then methyl iodide (4.79 g, 0.0337 mol) was added dropwise. The solution was removed to room temperature and after 1 h was added to 1 N HCl. The mixture was extracted with EtOAc to give crude *N*-acetyl-*N*-methyl-3,5-dichloroaniline, which was dissolved in THF (30 mL) and 6 N HCl (20 mL), and the solution was refluxed for 4 h. The cooled solution was basified with 1 N NaOH, and the mixture was extracted with EtOAc. The organic extract was washed with saturated $NaHCO_3$ and brine and dried over $MgSO_4$, and the solvent was removed by evaporation. The residue was purified by chromatography on silica gel to give *N*-methyl-5,7-dichloroaniline (5.05 g, 93% overall); ^1H NMR (60 MHz, $CDCl_3$) 2.7 (3 H, s, CH_3), 3.3 (1 H, bs, NH), and 6.35–6.7 (3 H, m, ArH). Conversion of this aniline to **66** (Table IX) was completed by reaction with diethyl acetylenedicarboxylate, as described above, followed by cyclization with polyphosphoric acid[43] at 100 °C and saponification.

5,7-Dichloro-4-methoxyquinoline-2-carboxylic Acid (67). 5,7-Dichloro-4-oxo-1,4-dihydroquinoline-2-carboxylic acid (**13**, 10 g, 0.0388 mol) was dissolved in phosphorus oxychloride (40 mL) and the solution heated at reflux for 1 h. The cooled solution was evaporated, and the residue was azeotroped with toluene (2 × 100 mL) and then refluxed with MeOH (100 mL) for 14 h. The MeOH was removed by evaporation, the residue triturated with CH_2Cl_2, and the resulting solid purified firstly by chromatography on silica gel, eluting with 1% MeOH in CH_2Cl_2, and then by crystallization from Et_2O/hexane to give methyl 5,7-dichloro-4-methoxyquinoline-2-carboxylate (2.15 g, 19%): mp 186–187 °C; ^1H NMR (360 MHz, DMSO-d_6) δ 3.96 (3 H, s, OCH_3), 4.10 (3 H, s, OCH_3), 7.58 (1 H, s, 3-H), 7.86 (1 H, d, 6-H), and 8.13 (1 H, d, 8-H); MS *m/e* 285 (M^+). Anal. ($C_{12}H_9Cl_2NO_3$) C, H, N. To a solution of this methyl ester (1 g, 0.0035 mol) in 65% aqueous methanol (150 mL) was added NaOH (0.56 g, 0.014 mol) and the solution refluxed for 2 h. The solution was acidified with 1 N HCl and the resulting precipitate collected and washed with warm water and dried to give the title compound (0.56 g, 59%, see Table IX).

1,6,7-Trimethyl-2,3-dioxo-1,2,3,4-tetrahydroquinoxaline (78). A solution of 4,5-dimethyl-*o*-phenylenediamine (5 g, 0.0367 mol) and 95% methyl glyoxalate (3.4 g, 0.0367 mol) in water (60 mL) and ethanol (60 mL) was heated under reflux for 2 h. The

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mixture was cooled and the precipitate that formed was collected and recrystallized from hot dimethylformamide (DMF) to give 6,7-dimethyl-2-oxo-1,2-dihydroquinoxaline (4.62 g, 72%): mp 303–306 °C; MS *m/e* 174 (M^+). Anal. ($C_{10}H_{10}N_2O$) C, H, N. To a solution of this quinoxaline (1.5 g, 0.00862 mol) and sodium hydride (55–60%, 0.42 g, 0.0175 mol) in DMF (75 mL) was added methyl iodide (1.37 g, 0.00964 mol). After 1.5 h the solution was evaporated to dryness and the residue washed with hexane and then recrystallized from MeOH/water to give the crude *N*-methyl derivative (1.42 g, 88%). A solution of this compound (1.15 g) in glacial HOAc (30 mL) and 30% hydrogen peroxide solution[31] (10 mL) was stirred at room temperature for 16 h. The resulting precipitate was collected and recrystallized from MeOH to give compound **78** (0.56 g, 45%; see Table IX).

6,7-Dimethyl-3-methoxy-2-oxo-1,2-dihydroquinoxaline (79). A solution of the quinoxalinedione **76** (3.72 g, 0.0196 mol) in phosphorus oxychloride (34 mL) was heated at 100 °C for 4 h. The cooled solution was evaporated to dryness and the residue washed with Et_2O to give 2,3-dichloro-6,7-dimethylquinoxaline (3.67 g, 83%), which was used without further purification. To a refluxing suspension of this dichloride (1.71 g, 0.00753 mol) in MeOH (100 mL) was added, over 2.5 h, a 0.30 N solution of NaOMe in MeOH (25 mL, 0.00761 mol). The mixture was refluxed for 1.75 h and cooled, and the precipitate was collected and purified by chromatography on silica gel to give 3-chloro-6,7-dimethyl-2-methoxyquinoxaline (0.97 g, 62%). A solution of this monochloride (0.92 g, 0.00446 mol) and KOH (1.6 g, 0.0286 mol) in water (25 mL) and MeOH (50 mL) was heated at reflux for 16 h, cooled, and filtered to afford compound **79** (0.59 g, 69%; see Table IX).

1,4,6,7-Tetramethyl-2,3-dioxo-1,2,3,4-tetrahydroquinoxaline (80). Methyl iodide (3.37 g, 0.0238 mol) was added to a solution of the quinoxalinedione **76** (2.06 g, 0.0118 mol) and sodium hydride (55–60%, 1.05 g, 0.024 mol) in DMF (100 mL). After 2 h water (100 mL) was added and the solid that resulted was collected and recrystallized from hot MeOH to provide pure **80** (1.13 g, 48%; see Table IX).

2,3-Dimethoxy-6,7-dimethylquinoxaline (81). To a solution of 2,3-dichloro-6,7-dimethylquinoxaline (1.63 g, 0.00718 mol) in MeOH (10 mL) was added a 1.44 N solution of NaOMe in MeOH (10 mL, 0.0144 mmol) and the mixture was refluxed for 4.5 h. After cooling, the precipitate was collected and recrystallized from MeOH to give the title compound (0.93 g, 59%; see Table IX).

^{13}C NMR Spectroscopic Investigation of Tautomerism in Kynurenic Acid and Quinoxalinedione. ^{13}C NMR spectra of the kynurenic acid derivatives **13**, **66**, and **67** and the quinoxalines **76** and **78–81** were obtained in DMSO.

(a) ^{13}C chemical shifts (ppm) of the kynurenates (C-3, C-6, and C-8 assignments were identified by one bond carbon/proton correlations and the remaining assignments by long range (COLOC) carbon/proton correlations). **13**: 143.30 or 138.39 (C-2), 112.89 (C-3), 176.53 (C-4), 120.68 (C-4a), 133.70 (C-5), 125.77 (C-6), 136.05 (C-7), 118.26 (C-8), 138.39 or 143.30 (C-8a), and 163.33 (CO_2H). **66**: 145.76 (C-2), 112.38 (C-3), 175.35 (C-4), 121.44 (C-4a), 134.10 (C-5), 126.21 (C-6), 136.84 (C-7), 116.76 (C-8), 144.72 (C-8a), 164.40 (CO_2H), and 38.79 (NCH_3). **67**: 151.64 or 149.95 (C-2), 102.44 (C-3), 163.93 (C-4), 117.89 (C-4a), 129.89 (C-5), 130.18 (C-6), 134.34 (C-7), 128.18 (C-8), 149.95 or 151.64 (C-8a), 165.80 (CO_2H), and 56.79 (OCH_3). The similar chemical shifts of C atoms in **13** and the *N*-methyl blocked analogue **66** suggest **13** exists predominantly in DMSO solution as the keto tautomer I.

(b) ^{13}C chemical shifts (ppm) of the quinoxalines. **76**: 155.0 (C-2/C-3), 130.93 (C-6/C-7), 123.20 (C-4a/C-8a), and 115.55 (C-5/C-8). **78**: 155.02, 153.40 (C-2/C-3), 131.47, 131.21 (C-6/C-7), 124.83, 123.62 (C-4a/C-8a), and 115.84, 115.59 (C-5/C-8). **80**: 153.55 (C-2/C-3), 131.76 (C-6/C-7), 124.79 (C-4a/C-8a), and 115.77 (C-5/C-8). **79**: 154.42 (C-2), 150.21 (C-3), 135.41, 131.45 (C-6/C-7), 128.16, 128.05 (C-4a/C-8a), 126.14 (C-5), and 115.08 (C-8). **81**: 149.08 (C-2/C-3), 135.77 (C-6/C-7), 134.73 (C-4a/C-8a), and 125.64 (C-5/C-8). The comparative chemical shifts at C-2/C-3 of **76**, **78**, and **79** with the doubly blocked compounds **80** and **81** clearly show that **76** and **78** exist as their 2,3-dione tautomers and **79** as the 2-keto tautomer.

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Registry No. **1**, 13593-94-7; **2**, 77474-31-8; **3**, 2379-57-9; **10**, 130063-95-5; **11**, 24796-59-6; **12**, 130063-96-6; **13**, 123158-59-8; **14**, 123158-04-3; **15**, 123157-83-5; **16**, 130063-97-7; **17**, 123157-92-6; **18**, 123157-86-8; **19**, 130096-01-4; **20**, 123157-89-1; **21**, 123158-01-0; **22**, 123157-95-9; **23**, 123158-12-3; **24**, 123157-98-2; **25**, 123158-24-7; **26**, 130063-98-8; **27**, 123158-27-0; **28**, 130063-99-9; **29**, 130064-00-5; **30**, 130064-01-6; **31**, 77474-34-1; **32**, 130064-02-7; **33**, 123158-33-8; **34**, 123158-07-6; **35**, 123158-15-6; **36**, 123158-21-4; **37**, 123158-18-9; **38**, 67085-16-9; **39**, 77474-33-0; **40**, 130064-03-8; **41**, 130064-04-9; **42**, 130064-05-0; **43**, 130064-06-1; **44**, 123157-57-3; **45**, 123173-47-7; **46**, 123157-56-2; **47**, 123157-58-4; **48**, 123157-71-1; **49**, 123157-63-1; **50**, 123157-62-0; **51**, 123157-65-3; **52**, 123157-66-4; **53**, 123157-60-8; **53** ethyl ester, 123158-45-2; **54**, 123157-61-9; **54** ethyl ester, 123158-44-1; **55**, 130064-07-2; **56**, 130064-08-3; **57**, 130064-09-4; **58**, 77474-29-4; **59**, 130064-10-7; **60**, 52980-05-9; **61**, 130064-11-8; **62**, 123158-30-5; **63**, 123158-10-1; **64**, 52980-06-0; **65**, 52980-08-2; **66**, 130064-12-9; **67**, 130064-13-0; **67** methyl ester, 130064-17-4; **68**, 42288-29-9; **69**, 130064-14-1; **70**, 39811-14-8; **71**, 15804-19-0; **72**, 127731-60-6; **73**, 6639-79-8; **74**, 4029-54-3; **75**, 25983-13-5; **76**, 2474-50-2; **77**, 2379-56-8; **78**, 4951-03-5; **79**, 130064-15-2; **80**, 4951-02-4; **81**, 130064-16-3; 4-chloro-2-nitroaniline, 89-63-4; 4-chloro-2-iodo-6-nitroaniline, 123158-75-8; 5-chloro-3-iodonitrobenzene, 123158-76-9; 3-chloro-5-iodoaniline, 83171-49-7; diethyl acetylenedicarboxylate, 762-21-0; 3,5-dichloroaniline, 626-43-7; *N*-methyl-3,5-dichloroaniline, 42266-03-5; 4,5-dimethyl-*o*-phenylenediamine, 3171-45-7; methyl glyoxalate, 922-68-9; 6,7-dimethyl-2-oxo-1,2-dihydroquinoxaline, 28082-82-8; 2,3-dichloro-6,7-dimethylquinoxaline, 63810-80-0; *N*-methyl-D-aspartic acid, 6384-92-5.