6-(1*H*-Imidazol-1-yl)-7-nitro-2,3(1*H*,4*H*)-quinoxalinedione Hydrochloride (YM90K) and Related Compounds: Structure–Activity Relationships for the AMPA-Type Non-NMDA Receptor

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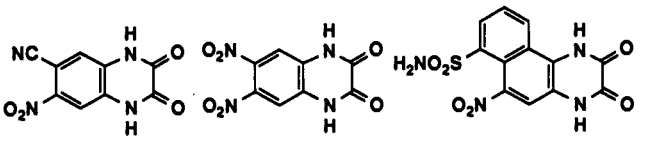
A novel series of quinoxalinediones possessing imidazolyl and related heteroaromatic substituents was synthesized and evaluated for their activity to inhibit [^{3}H]AMPA binding from rat whole brain. From the structure–activity relationships, it was found that the 1*H*-imidazol-1-yl moiety could function as a bioisostere for the cyano and nitro groups, and that 6-(1*H*-imidazol-1-yl)-7-nitro-2,3(1*H*,4*H*)-quinoxalinedione (**11**) showed the most potent activity for the AMPA receptor. Compound **11** was evaluated for selectivity versus other excitatory amino acid receptors, and its action against AMPA at its receptor in the rat striatum was characterized. These data showed that compound **11** was a selective antagonist for the AMPA receptor with a K_i value of 0.084 μM, being approximately equipotent with 2,3-dihydro-6-nitro-7-sulfamoylbenzo(*f*)quinoxaline (**3**) (NBQX; K_i = 0.060 μM). Compound **11** was also found to give protection against sound-induced seizure on DBA/2 mice at the minimum effective dose of 3 mg/kg ip (**3**; 10 mg/kg ip).

L-Glutamate is a major excitatory amino acid (EAA) neurotransmitter in the mammalian central nervous system.[1-3] Since overstimulation of EAA receptors has been linked to a number of neurodegenerative disorders such as ischemia, stroke, and epilepsy, as well as Huntington's and Alzheimer's diseases, there has been growing interest in the potential therapeutic value of EAA antagonists.[4,5] Postsynaptic EAA receptors have been subdivided into four main subtypes,[6] namely the *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA) (renamed from the quisqualate receptor), kainate (KA), and metabotropic glutamate receptor subtypes. AMPA and KA receptors may be grouped collectively as non-NMDA receptors.[7] Of these four subtypes, the NMDA receptor subtype has been well studied because of the availability of both competitive and noncompetitive antagonists. Although NMDA antagonists have shown cerebroprotective effects in a focal ischemia model,[8] their effects were inconclusive in a global ischemia model.[9,10] Furthermore, these antagonists produce a psychotomimetic action,[11] an impairment of learning behavior,[12] and ultrastructural changes in cortical neurons[13] that may limit their use as therapeutic agents.

There has been considerable recent interest in selective antagonists for the AMPA receptor, such as 6-cyano-7-nitroquinoxaline-2,3-dione (**1**) (CNQX), 6,7-dinitroquinoxaline-2,3-dione (**2**) (DNQX),[14] and 2,3-dihydro-6-nitro-7-sulfamoylbenzo(*f*)quinoxaline (**3**),[15] because of their physiological and pharmacological activity.[16,17] These disclosures have prompted an intensive search for more potent and selective AMPA antagonists in order to explore the role of this receptor in more detail and to address their therapeutic potential in a number of CNS disorders. In this paper, we describe the design, synthesis, and structure–activity relationships of 6-(1*H*-imidazol-1-yl)-7-nitro-2,3-(1*H*,4*H*)-quinoxalinedione (**11**) and related compounds.



1 (CNQX) **2** (DNQX) **3** (NBQX)

Figure 1.

Chemistry

Most of the imidazole derivatives were prepared directly by displacement of halides from the appropriate nitrohalobenzenes. Three sets of conditions were used to accomplish this transformation: by generation of the sodio anion of imidazole with NaH in anhydrous DMF followed by reaction with the appropriate fluorobenzene; by simply refluxing the fluorobenzene with a 3–5 molar excess of imidazole in anhydrous DMF; and by heating the chlorobenzene with an excess of imidazole and potassium hydroxide in anhydrous DMSO. Synthesis of the desired quinoxalinediones was accomplished as shown in Schemes 1–4.

Synthesis of 6-(1*H*-imidazol-1-yl)-7-nitro-2,3(1*H*,4*H*)-quinoxalinedione (**11**) is shown in Scheme 1. Reaction of compound **8** with excess imidazole and potassium hydroxide in DMSO at 80 °C gave 5-(1*H*-imidazol-1-yl)-2-nitroaniline (**9**). Hydrogenation of this nitro derivative with palladium on carbon followed by treatment with oxalic acid in refluxing 4 N HCl gave the corresponding imidazolylquinoxalinedione **10**. Nitration of **10** with $^fHNO_3$ in concentrated H_2SO_4 at room temperature gave the desired compound **11**. The 8-nitro isomer **12**, formed as a byproduct of this reaction, was easily separated by recrystallization from H_2O.

The versatile intermediate **15** was prepared in two steps from 4-fluoro diamine **13** as illustrated in Scheme 2. This intermediate **15** was treated with several azoles to give the desired compounds **16–24** (see Table 2).

The 4(C)-imidazolyl derivative **30a** was prepared from 4-phenylimidazole (**25a**) (Scheme 3). The readily available

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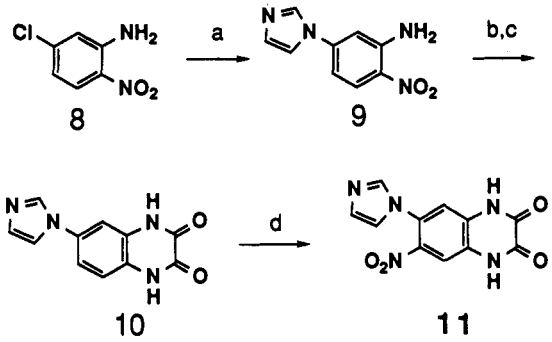
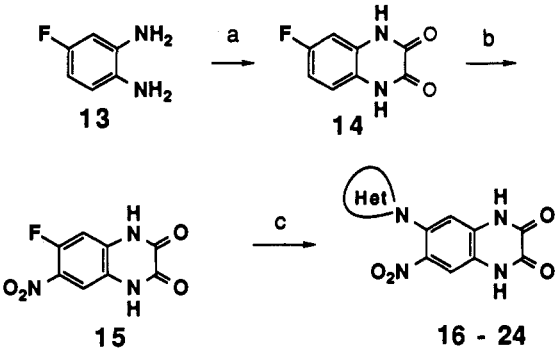
Scheme 1[a]



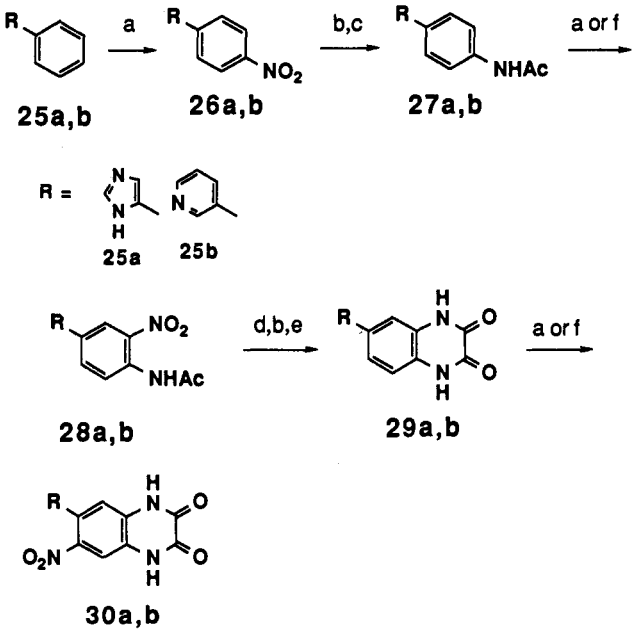
[a] (a) Imidazole, KOH, DMSO; (b) H_2, Pd–C (10%), HCl; (c) $(COOH)_2$, 4 N HCl; (d) $^fHNO_3$, H_2SO_4.

Scheme 2[a]



[a] (a) $(COOH)_2$, 4 N HCl; (b) KNO_3, H_2SO_4; (c) azoles.

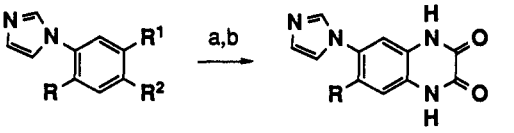
Scheme 3[a]



[a] (a) $^fHNO_3$, H_2SO_4; (b) H_2, Pd–C; (c) Ac_2O, Et_3N, $CHCl_3$; (d) HCl; (e) $(COOH)_2$, 4 N HCl; (f) NO_2BF_4, tetramethylene sulfone.

acetamide **27a**, prepared by a three-step sequence from **25a**, was nitrated with nitronium tetrafluoroborate[18] in tetramethylene sulfone to give *o*-nitro acetamide **28a**. When we attempted this transformation using $^fHNO_3$ or KNO_3 in concentrated H_2SO_4, the reaction did not proceed to completion. Acidic hydrolysis gave the corresponding nitroaniline, which was hydrogenated and then treated with oxalic acid to give quinoxalinedione **29a**. Finally, this quinoxalinedione was nitrated in a sequence similar to that for **27a** to give the desired compound **30a**. The

Scheme 4[a]



R^1, R^2 = NO_2, NH_2

[a] (a) H_2, Pd–C (10%), 1 N HCl; (b) $(COOH)_2$, 4 N HCl.

3-pyridyl analog **30b** was prepared from the corresponding **25b** in a similar manner except that the nitration of **27b** and **29b** utilized $^fHNO_3$ in concentrated H_2SO_4.

The various 6-imidazolyl-7-substituted derivatives **45–50** (see Table 3) were prepared from the corresponding imidazolylnitroanilines as shown in Scheme 4. Synthesis of each substituted imidazolylnitroaniline is outlined in Schemes 5 and 6.

The 2-fluoro-5-nitro derivatives **31a–c** were converted to the corresponding acetamides **33a–c** after displacement of the fluoride with imidazole (Scheme 5). The methyl, **33a**, and fluoro, **33b**, derivatives were treated with $^fHNO_3$ in concentrated H_2SO_4 followed by hydrolysis to give *o*-nitro acetamides **35a** and **35b**, respectively. Substitution reaction of the fluoro derivative **34b** with imidazole followed by hydrolysis gave the 6,7-diimidazole derivative **36**.

As nitration of the trifluoromethyl derivative **33c** did not gave the desired compound in spite of treatment under several reaction conditions, we attempted to switch the order of steps so that the substitution reaction of imidazole took place after nitration. Hydrogenation of **31c** followed by acetylation of the corresponding aniline gave fluoro acetamide **37**, which was nitrated in the usual manner to give the 1,2,4,5-substituted derivative **38** (84%) and the 1,2,3,4-substituted derivative **39** (8.6%), the structures of which were determined by ^1H NMR (see the Experimental Section). Treatment of **38** and **39** with imidazole followed by hydrolysis gave the imidazolyl derivatives **35c** and **40**, respectively.

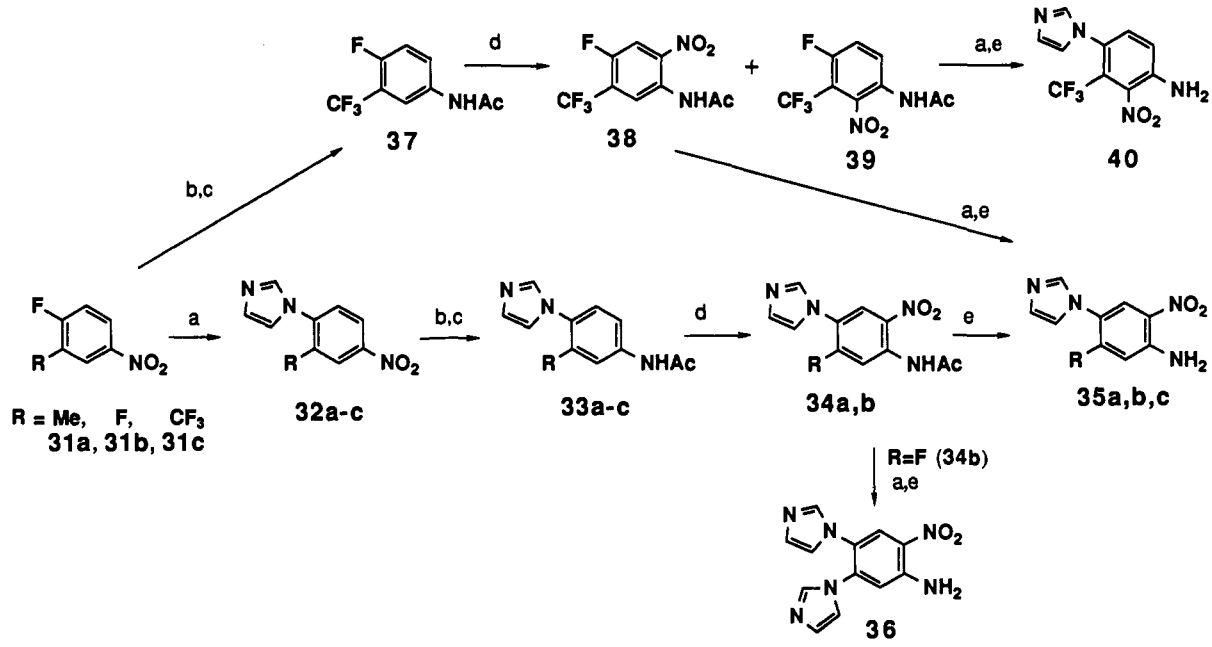
The cyano, **44a**, and acetyl, **44b**, derivatives were obtained from the appropriate 2,4-difluorobenzenes **41a** and **41b** (Scheme 6). Compound **41a** was nitrated in hot $^fHNO_3$ (80 °C) to successfully produce 5-nitrobenzonitrile **42a**, which was treated with ammonia to give *o*-nitroaniline **43a**. Treatment of **43a** with imidazole gave the desired compound **44a**. A similar sequence of reactions with acetophenone **41b** gave the corresponding acetyl derivative **44b**.

Results and Discussion

The compound structures and results of the radioreceptor assay for AMPA[19] are summarized in Tables 1–3.

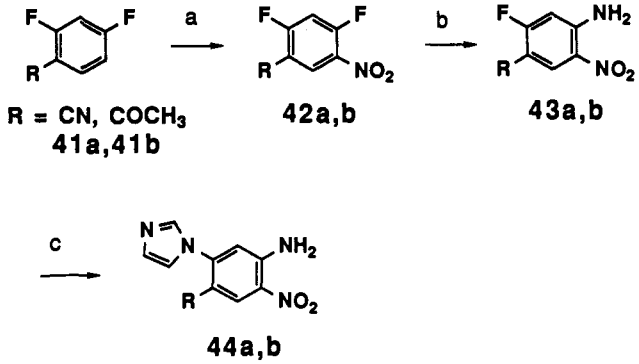
Quinoxaline-2,3-dione (**4**) and the 6-methyl derivative **5** had only weak affinity for the AMPA receptor (Table 1). In contrast, cyano, **6**, and nitro, **7**, substitutions at the 6-position resulted in enhanced potencies, with K_i values of 5.0 and 2.0 μM, respectively. In order to discover new bioisosteres for the cyano and nitro groups, we focused on the planarity with the π-bond system and hydrophilicity of these groups. First, compound **10** bearing an N-linked imidazole ring was prepared. The planarity of the imidazole ring is similar to that of the cyano group and, particularly, the nitro group with regard to the extended structure. The hydrophilicity of the imidazole ring (π = –0.65) is almost the same as that of the CN group (π =

Scheme 5[a]



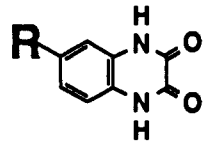
[a] (a) Imidazole, DMF; (b) H_2, Pd–C (10%), EtOH; (c) Ac_2O, Et_3N, $CHCl_3$; (d) $^fHNO_3$, H_2SO_4; (e) HCl.

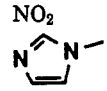
Scheme 6[a]



[a] (a) $^fHNO_3$; (b) aqueous NH_3, EtOH; (c) imidazole, DMF.

Table 1. 6-Substituted Quinoxalinedione Derivatives



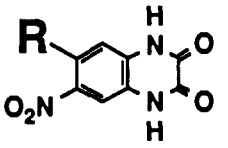
compd	R	AMPA receptor affinity K_i (μM)[a]
4	H	24% (100)[b]
5	Me	28% (100)[b]
6	CN	5.0 (4.9–5.0)
7	NO_2	2.0 (2.0–2.1)
10		1.6 (1.6–1.7)

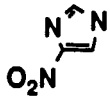
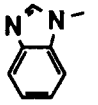
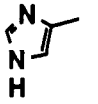
[a] K_i values were determined by double experiments performed in triplicate. Values in parentheses are 95% confidence intervals. [b] These compounds were tested at the concentration (μM) indicated with the percent inhibition of binding shown.

–0.57) and slightly polar related to that of the nitro group (π = –0.28).[20,21] This transformation resulted in binding activity (K_i = 1.6 μM) nearly equal to those of **6** and **7**, respectively. As the presence of a nitro group at the 7-position of **1** and **2** seems to be essential for the enhancement of their inhibitory activity for the AMPA receptor, a compound with a nitro group at the 7-position of the 6-(1*H*-imidazol-1-yl) derivative **10** was synthesized to confirm this possibility. As predicted, the desired compound, 6-(1*H*-imidazol-1-yl)-7-nitro-2,3(1*H*,4*H*)-quinoxalinedione (**11**), exhibited markedly potent activity, with the K_i value of 0.084 μM indicating over 1000-fold improved affinity related to that of the parent quinoxaline-2,3-dione, 2 to 3 times higher affinity than those of **1** and **2** (Table 2), and approximately similar affinity to that of **3** [K_i = 0.060 μM (lit.[15] = 0.15 μM)]. It was found that the imidazole ring is nonionic at physiological pH (7.4) from the values of the measured pK_a of compound **11** [N^3-(4.7), 1-NH (10.8), and 4-NH (7.4)].[22,23] These physicochemical properties and the binding data indicate that the 1*H*-imidazol-1-yl ring can function as a new bioisostere for the cyano and nitro groups in the binding of these compounds to the AMPA receptor. To the best of our knowledge, this finding has not been previously reported. These results prompted us to investigate in more detail the structure–activity relationships of the imidazolyl quinoxalinediones. Holding the 7-nitro substitution pattern constant, we proceeded with examining the effects of substituted imidazoles and other heteroaromatics at the 6-position. The data for these derivatives are shown in Table 2. Since substitutions of methyl, **18**, phenyl, **19**, and nitro, **20**, at the 4-position of the imidazole ring of compound **11** led to only modest decreases in potency, it seemed that there was a pocket at this site of the receptor able to tolerate steric hindrance. In contrast, 2-substituted imidazole derivatives **16** and **17** showed 5-fold less activities related to that of compound **11**, while 4,5-fused derivatives **21** and **22** showed marked decreases.

The 1*H*-1,2,4-triazol-1-yl derivative **23**, which has a nitrogen at the 5-position of the imidazole, showed relatively good potency (K_i = 0.24 μM). However, removal of the N^4-nitrogen of the 1,2,4-triazol-1-yl ring to give the pyrazole derivative **24** caused a drastic reduction in potency. Replacement of the N-linked imidazole in structure **11** by a 4(C)-linked imidazole to give **30a** decreased potency 8-fold. Compound **30b** possessing a 3-pyridyl ring also showed reduced activity. The precise positioning of the nitrogen atoms in the heterocyclic ring and the specific electronic topography of the heterocyclic

Table 2. 7-Nitro-6-substituted Quinoxalinedione Derivatives



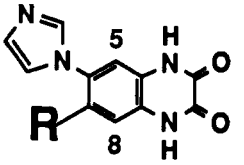
compd	R	AMPA receptor affinity K_i (μM)[a]
1	CN	0.27 (0.26–0.27)
2	NO_2	0.20 (0.19–0.21)
11		0.084 (0.083–0.086)
16		0.43 (0.41–0.45)
17		0.39 (0.35–0.43)
18		0.18 (0.17–0.19)
19		0.23 (0.21–0.24)
20		0.10 (0.091–0.12)
21		4.8 (4.5–5.2)
22		1.3 (1.3–1.4)
23		0.24 (0.23–0.25)
24		1.4 (1.3–1.4)
30a		0.69 (0.60–0.78)
30b		0.62 (0.59–0.66)

[a] See Table 1.

rings are both important for significant binding to the AMPA receptor.

We next turned our attention to explore the effects of substitutions at the 7-position on receptor affinity. The highest affinities resided with the cyano, **45**, and trifluoromethyl, **46**, derivatives (K_i values 0.20 μM, see Table 3), suggesting that increasing potency is associated with increasing electron-withdrawing ability. The reduced activity of the acetyl derivative **47** in spite of its relatively strong electron-withdrawing ability may be a consequence of the strictly size-limited site at the 7-position. The methyl, **49**, and amino, **51**, derivatives, bearing electron-donating groups, and the fluoro derivative **48**, which has a weak electron-withdrawing group, showed significant decreases in activity. The replacement of both nitro groups of **2** with imidazoles to give the 6,7-diimidazole derivative

Table 3. 6-(1*H*-Imidazol-1-yl)quinoxalinedione Derivatives



compd	R	AMPA receptor affinity K_i (μM)[a]
45	CN	0.20 (0.19–0.20)
46	CF_3	0.20 (0.20–0.21)
47	$COCH_3$	36% (1.0)[b]
48	F	14% (1.0)[b]
49	Me	14% (1.0)[b]
50		0.82 (0.80–0.85)
51	NH_2	16% (1.0)[b]
12	8-NO_2	1.9 (1.9–2.0)
52	5-CF_3	10% (1.0)[b]

[a,b] See Table 1.

50 led to 4-fold less activity than that of **2** and 10 times decreased affinity related to that of compound **11**. The second imidazole moiety seems to be a poor bioisostere for the nitro group, and the result suggests the existence of steric hindrance at the 7-position as in the case of the acetyl derivative **47**. Moving the nitro group of **11** to the 8-position to give compound **12** and the trifluoromethyl group of **46** to the 5-position to give compound **52** resulted in diminished activity, indicating that the optimal position of the electron-withdrawing group is the 7-position.

Among the compounds prepared in this series, 6-(1*H*-imidazol-1-yl)-7-nitro-2,3(1*H*,4*H*)-quinoxalinedione (**11**) showed the most potent activity for AMPA binding and was selected for further study.

Table 4 shows the inhibitory activity of compounds **1**, **3**, and **11** for some excitatory amino acid receptor subtypes (KA,[24] NMDA-sensitive glutamate,[25] and strychnine-insensitive glycine[26]). Compound **11** showed no inhibitory activity for the NMDA receptor subtype. Affinities of this compound for the kainate and glycine receptors were about 20 and 400 times less than that for the AMPA receptor, the K_i values being 2.2 and 37 μM, respectively. Binding activity of compound **11** for the AMPA receptor was more selective than that of **1**. Compounds reported to possess glycine/NMDA antagonist activity include quinoxaline-2,3-diones and kynurenic acids.[27,28] One of the structural features required for this activity is small (usually hydrophobic) 5-, 6-, and 7-substituents.[22] Introduction of the bulkier and hydrophilic imidazole ring in the quinoxaline-2,3-dione results in a great decrease in activity at both the glycine and NMDA sites and produces potent selectivity for the AMPA site.

Compound **11** was characterized in vivo by its ability to prevent a decrease in the activity of choline acetyltransferase (ChAT) produced by AMPA in the rat striatum (see Figure 2). AMPA (25 nmol) caused a 40–55% decline in ChAT activity at 7 days after intrastriatal injection. Coinjection of compound **11** protected rat striatum from this damage in a dose-dependent manner. This result indicates that compound **11** has an antagonistic action against AMPA at its receptor in the striatum.

Testing of compound **11** in the DBA/2 mouse model[29] showed the most potent anticonvulsant activity among selected compounds following systemic (ip) administration (minimum effective dose (MED) was 3 mg/kg when administered at 15 min prior to sound exposure: **1**, MED

Table 4. Affinities of 1, 3, and 11 for Various Glutamate Receptor Subtypes

	glutamate receptor subtypes			
	AMPA receptor	kainate receptor	NMDA receptor–ion channel complex	
		high-affinity site	NMDA-binding site	strychnine-insensitive glycine site
	ligand K_i (μM)[a]			
	[^{3}H]AMPA	[^{3}H]kainate	[^{3}H]glutamate	[^{3}H]glycine
1	0.27	1.8 (1.8–1.9)	25 (23–26)	5.6 (4.9–6.4)
3	0.060 (0.058–0.061)	4.1 (3.9–4.3)	>100	>100
11	0.084	2.2 (2.1–2.2)	>100	37 (34–40)

[a] See Table 1.

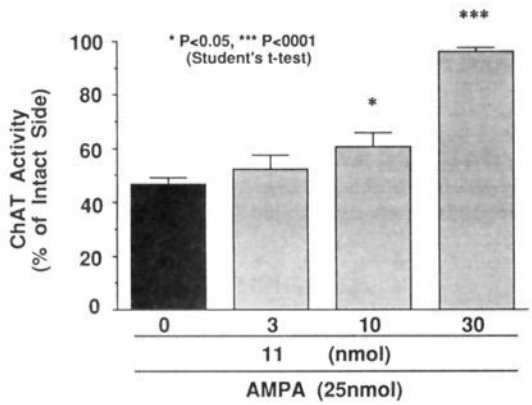


Figure 2. Dose-dependent effects of compound 11 on AMPA-induced striatal neurodegeneration in rats. The extent of neuronal damage was assessed by determination of the activity of ChAT present in interneurons found in the striatum. The abscissa indicates the nanomole of agonists, and the ordinate indicates the percentage of ChAT activity in the lesioned striatum relative to the contralateral (nonlesioned) striatum. Vertical bars represent the mean ± S.E. of three to four rats.

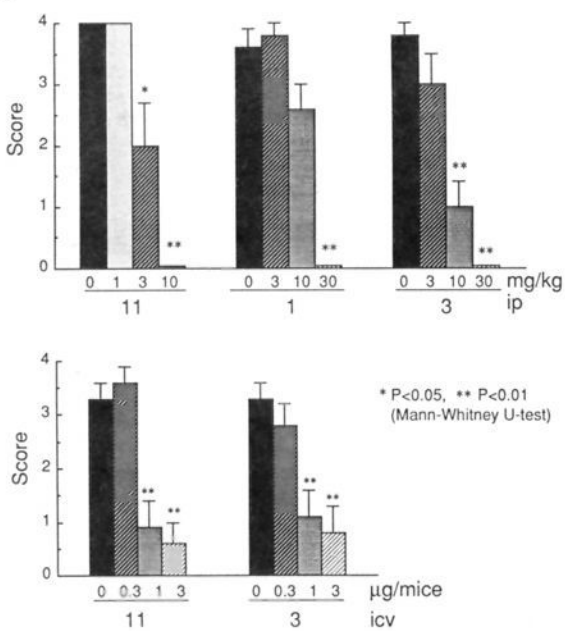


Figure 3. Anticonvulsant effects of 1, 3, and 11 on sound-induced seizure in DBA/2 mice.

= 30 mg/kg, and 3, MED = 10 mg/kg) (Figure 3). On the other hand, activity following icv dosing showed a correlation between affinity for AMPA (K_i values) and icv anticonvulsant potency (MED: 11, 1 μg (3.6 nmol)/mouse, and 3, 1 μg (3.0 nmol)/mouse). These results show that the permeability of 11 into the brain is somewhat better than that of 3.[30] Compound 11 also exhibited neuroprotective activities against both delayed neuronal death in a gerbil global ischemic model and cerebral infarction in a rat focal ischemia model on postischemic treatment.[10,31,32] A more detailed account of this research will be reported elsewhere.

In summary, we have shown that the 1*H*-imidazol-1-yl moiety is an efficient bioisostere for the cyano and nitro groups in compounds binding to the AMPA receptor. Among this series of quinoxalinediones possessing imidazolyl and related heteroaromatic substituents, 6-(1*H*-imidazol-1-yl)-7-nitro-2,3(1*H*,4*H*)-quinoxalinedione hydrochloride (11·HCl) (YM90K; formerly YM900[10,31–34]) was found to be a potent and selective antagonist for the AMPA-type non-NMDA EAA receptor and may represent a candidate for development as a therapeutic agent for the treatment of a number of CNS disorders.

Experimental Section

Chemistry. Melting points were measured on a Yanaco MP-3 melting point apparatus and are uncorrected. Unless stated otherwise, ^{1}H NMR spectra were measured in DMSO with either a JEOL FX90Q or FX 100 spectrometer; chemical shifts are expressed in δ units using tetramethylsilane as the standard (in NMR description, s = singlet, d = doublet, t = triplet, q = quartet, and m = multiplet). Mass spectra were recorded with a Hitachi M-80 or JEOL JMS-DX300 spectrometer. Where elemental analyses (C,H,Cl,F,N,S) are indicated only by symbols of the elements, analytical results obtained for these elements were within ±0.4% of theoretical values except where otherwise stated. All solutions were dried over anhydrous magnesium sulfate, and the solvent was evaporated under reduced pressure.

6-(1*H*-Imidazol-1-yl)-2,3(1*H*,4*H*)-quinoxalinedione Hydrochloride (10·HCl). A solution of 8 (10 g, 58.0 mmol), KOH (85%, 5.6 g, 85.0 mmol), and imidazole (15.7 g, 230 mmol) in DMSO (50 mL) was heated at 80 °C for 3 h and then poured onto ice–water. The resultant precipitate was collected by filtration and washed with water to yield 9 (11.1 g, 54.4 mmol, 94%), which was hydrogenated in 1 N HCl (40 mL) at atmospheric pressure using 10% palladium on carbon (Pd–C, 900 mg) as the catalyst in 1 N HCl (40 mL) to give the diamine. This diamine was treated with oxalic acid (7.3 g, 81 mmol) in 4 N HCl (50 mL) at reflux overnight. The resulting precipitate was collected and washed with water to give 10·HCl (7.89 g, 54%): mp >300 °C; ^{1}H NMR δ 12.33 (br s, 1 H), 12.27 (br s, 1 H), 9.65 (t, J = 1.5 Hz, 1 H), 8.16 (t, J = 1.5 Hz, 1 H), 7.91 (t, J = 1.5 Hz, 1 H), 7.45 (m, 3 H), 7.01 (br 1 H); MS (EI) m/z 228 (M). Anal. ($C_{11}H_8N_4O_2$·HCl·0.1H_2O) C,H,Cl,N.

6-(1*H*-Imidazol-1-yl)-7-nitro-2,3(1*H*,4*H*)-quinoxalinedione Hydrochloride (11·HCl) and 6-(*H*-Imidazol-1-yl)-8-nitro-2,3(1*H*,4*H*)-quinoxalinedione (12). To a solution of 10 (6.5 g, 24.6 mmol) in H_2SO_4 (30 mL) was added ^{f}HNO$_3$ (d = 1.52, 1.12 mL, 27.0 mmol) dropwise below 20 °C, and the reaction mixture was stirred at room temperature for 2 h. The reaction mixture was poured onto ice–water and neutralized with aqueous sodium hydroxide. The resulting precipitate was mixed with 4 N HCl (40 mL), heated at reflux for 0.5 h, cooled to room temperature, and then filtered. Recrystallization of the solid from water (100 mL) gave the HCl salt of 11 (4.09 g, 53.7%) after removing the insoluble product in hot water: mp >300 °C; ^{1}H NMR δ 8.68 (s,

1 H), 8.02 (s, 1 H), 7.82 (s, 1 H), 7.50 (s, 1 H), 7.28 (s, 1 H); MS (FAB) m/z 274 (M + 1). Anal. ($C_{11}H_7N_5O_4$·HCl) C,H,Cl,N.

The insoluble product in hot water was washed several times with hot diluted HCl to give **12**: mp >300 °C; ^{1}H NMR (D_2O–K_2CO_3) δ 8.03 (t, J = 1.0 Hz, 1 H), 7.60 (d, J = 2.6 Hz, 1 H), 7.46 (m, 1 H), 7.37 (d, J = 2.6 Hz, 1 H), 7.14 (m, 1 H); MS (FAB) m/z 274 (M + 1). Anal. ($C_{11}H_7N_5O_4$·0.4H_2O) C,H,N.

6-Fluoro-7-nitro-2,3(1*H*,4*H*)-quinoxalinedione (15). A solution of 4-fluoro diamine **13** (9.10 g, 72.2 mmol) and oxalic acid (13 g, 14.4 mmol) in 4 N HCl (90 mL) was refluxed for 4 h, cooled, and filtered to give **14** (12.3 g, 94%). To a solution of **14** (5.3 g, 29.4 mmol) in concentrated H_2SO_4 (53 mL) was carefully added KNO_3 (3.27 g, 32.3 mmol) at 0–5 °C. The solution was stirred at room temperature for 2 h and poured onto ice–water to give solid **15** (3.62 g, 54.6%): mp >300 °C (DMF–H_2O); ^{1}H NMR δ 12.36 (s, 1 H), 12.09 (s, 1 H), 7.84 (d, J = 7.2 Hz, 1 H), 7.06 (d, J = 12.1 Hz, 1 H); MS (FAB) m/z 226 (M + 1). Anal. ($C_8H_4FN_3O_4$·0.5DMF) C,H,N.

General Method for Preparation of 6-Substituted-7-nitro-2,3(1*H*,4*H*)-quinoxalinediones 16–24. A solution of **15** and the appropriate azole (3–5 equiv mol) with or without base in tetramethylene sulfone, DMF, or neat was heated at 140–150 °C for 2–5 h and then poured into water to give the corresponding quinoxalinediones.

6-(1*H*-2-Methylimidazol-1-yl)-7-nitro-2,3(1*H*,4*H*)-quinoxalinedione (16): 61% from **15**; mp >300 °C (DMF–H_2O); ^{1}H NMR δ 12.42 (br s, 2 H), 7.95 (s, 1 H), 7.19 (d, 1 H), 7.12 (s, 1 H), 6.93 (d, 1 H), 2.09 (s, 3 H, Me); MS (m/z) 288 (M + 1). Anal. ($C_{12}H_9N_5O_4$·1.3H_2O) C,H,N.

6-(1*H*-2-Ethylimidazol-1-yl)-7-nitro-2,3(1*H*,4*H*)-quinoxalinedione (17): 90% from **15**; mp >300 °C; ^{1}H NMR δ 12.01 (br s, 2 H), 7.94 (s, 1 H), 7.18 (d, J = 1.5 Hz, 1 H), 7.11 (s, 1 H), 6.95 (d, J = 1.5 Hz, 1 H), 2.38 (q, J = 7.5 Hz, 2 H), 1.09 (t, J = 7.5 Hz, 3 H); MS (m/z) 302 (M + 1). Anal. ($C_{13}H_{11}N_5O_4$·H_2O) C,H,N.

6-(1*H*-4-Methylimidazol-1-yl)-7-nitro-2,3(1*H*,4*H*)-quinoxalinedione (18): 60% from **15**; mp >300 °C; ^{1}H NMR δ 12.39 (br s, 2 H), 7.85 (s, 1 H), 7.71 (d, 1 H), 7.08 (s, 1 H), 7.04 (d, 1 H), 2.15 (s, 3 H); MS (m/z) 288 (M + 1). Anal. ($C_{12}H_9N_5O_4$·0.2H_2O) C,H,N.

6-(1*H*-4-Phenylimidazol-1-yl)-7-nitro-2,3(1*H*,4*H*)-quinoxalinedione (19): 40% from **15**; mp >300 °C; ^{1}H NMR δ 12.45 (s, 1 H), 12.30 (s, 1 H), 7.96–7.94 (3 H), 7.83 (s, 1 H), 7.82 (s, 1 H), 7.39 (2 H), 7.25 (s, 1 H), 7.21 (s, 1 H); MS (m/z) 350 (M + 1). Anal. ($C_{17}H_{11}N_5O_4$·0.6H_2O) C,H,N.

6-(1*H*-4-Nitroimidazol-1-yl)-7-nitro-2,3(1*H*,4*H*)-quinoxalinedione (20): 25% from **15**; mp >300 °C; ^{1}H NMR δ 12.55 (s, 1 H), 12.32 (s, 1 H), 8.80 (d, 1 H), 8.20 (d, 1 H), 8.04 (s, 1 H), 7.30 (s, 1 H); MS (m/z) 319 (M + 1). Anal. ($C_{11}H_6N_6O_6$) C,H,N.

6-(1*H*-4,5,6,7-Tetrahydrobenzimidazol-1-yl)-7-nitro-2,3-(1*H*,4*H*)-quinoxalinedione (21): 44% from **15**; mp >300 °C; ^{1}H NMR δ 12.35 (br s, 2 H), 7.96 (s, 1 H), 7.79 (s, 1 H), 7.15 (s, 1 H), 2.50 (m, 2 H), 2.18 (m, 2 H), 1.70 (m, 2 H); MS (m/z) 328 (M + 1). Anal. ($C_{15}H_{13}N_5O_4$·1.5H_2O) C, H, N.

6-(1*H*-Benzimidazol-1-yl)-7-nitro-2,3(1*H*,4*H*)-quinoxalinedione (22): 12% from **15**; mp >300 °C; ^{1}H NMR δ 12.37 (br s, 2 H), 8.45 (s, 1 H), 8.05 (s, 1 H), 7.78 (m, 1 H), 7.29 (4 H); MS (m/z) 324 (M + 1). Anal. ($C_{15}H_9N_5O_4$·1.2H_2O) C, H, N.

6-(1*H*-1,2,4-Triazol-1-yl)-7-nitro-2,3(1*H*,4*H*)-quinoxalinedione (23): 71% from **15**; mp >300 °C; ^{1}H NMR δ 12.40 (br s, 2 H), 9.02 (s, 1 H), 8.25 (s, 1 H), 7.88 (s, 1 H), 7.49 (s, 1 H); MS (m/z) 275 (M + 1). Anal. ($C_{10}H_6N_6O_4$·0.4H_2O) C, H, N.

6-(1*H*-Pyrazol-1-yl)-7-nitro-2,3(1*H*,4*H*)-quinoxalinedione (24): 18% from **15**; mp >300 °C; ^{1}H NMR δ 12.28 (br s, 2 H), 8.17 (d, J = 2 Hz, 1 H), 7.75 (s, 1 H), 7.72 (d, 1 H), 7.25 (s, 1 H), 6.54 (t, J = 2 Hz, 1 H); MS (m/z) 274 (M + 1). Anal. ($C_{11}H_7N_5O_4$), C, H, N.

4-(3-Pyridyl)acetanilide (27b). To an ice-cold solution of **25b** (10 g, 64.5 mmol) in H_2SO_4 (50 mL) was added ^{f}HNO$_3$ (2.80 mL, 67.6 mmol), and the resulting mixture was stirred at that temperature for 1 h. The solution was poured onto ice (500 g) and neutralized with aqueous NaOH. The resulting precipitate was collected and recrystallized from methanol to give **26b** (7.0 g, 54%): mp 185–186 °C; ^{1}H NMR δ 8.98 (dd, 1 H), 8.66 (dd, 1 H), 7.97–8.96 (5 H), 7.55 (ddd, 1 H).

A solution of **26b** (6.81 g, 34.1 mmol) in methanol (40 mL) and concentrated HCl (6 mL) was hydrogenated at atmospheric pressure using Pd–C (10%, 0.34 g) as catalyst. After filtration, the solution was evaporated and treated with acetic anhydride (3.53 mL, 35.0 mmol) and triethylamine (15.6 mL, 107 mmol) in methylene chloride (80 mL) at room temperature. The resulting precipitate was filtered off and recrystallized from methanol–H_2O to give **27b** (6.32 g, 87%): ^{1}H NMR δ 10.08 (s, 1 H), 8.87 (br s, 1 H), 8.55 (d, 1 H), 8.04 (br s, 1 H), 7.71 (m, 4 H), 7.45 (ddd, 1 H), 2.09 (s, 3 H); MS (EI) m/z 212 (M).

4-(3-Pyridyl)-2-nitroacetanilide (28b). To an ice-cold solution of **27b** (5.0 g, 23.6 mmol) in H_2SO_4 (50 mL) was added ^{f}HNO$_3$ (1.03 mL, 24.9 mmol), and the reaction mixture was stirred at room temperature for 1 h. The solution was poured onto ice (500 g), neutralized with aqueous NaOH, and filtered. The product was recrystallized from methanol–H_2O to give **28b** (4.29 g, 71%): mp 162–164 °C; ^{1}H NMR δ 10.36 (s, 1 H), 8.97 (br s, 1 H), 8.63 (br s, 1 H), 8.03–8.28 (m, 3 H), 7.75 (d, 1 H), 7.52 (dd, 1 H), 2.11 (s, 3 H); MS (EI) m/z 257 (M).

6-(1*H*-Imidazol-4-yl)-2,3(1*H*,4*H*)-quinoxalinedione Hydrochloride (29a·HCl): 54% (three steps from 4-(1*H*-imidazol-4-yl)-2-nitroacetanilide **28a**[35]); mp >300 °C; ^{1}H NMR δ 14.5 (br s, 1 H), 12.22 (s, 1 H), 12.17 (s, 1 H), 9.23 (d, 1 H), 8.01 (d, 1 H), 7.50–7.66 (m, 2 H), 7.28 (d, 1 H); MS (FAB) m/z 229 (M + 1). Anal. ($C_{11}H_8N_4O_2$·HCl·1.2H_2O) C, H, Cl, N.

6-(1*H*-Imidazol-4-yl)-7-nitro-2,3(1*H*,4*H*)-quinoxalinedione Hydrochloride (30a·HCl). A solution of **29a** (0.43 g, 1.89 mmol) and nitronium tetrafluoroborate (0.43 g, 3.24 mmol) in tetramethylene sulfone (4 mL) was heated at 120 °C for 2 h. The solution was poured onto ice–water and neutralized with 1 N NaOH. The solid was suspended in ethanol and added to 4 N HCl in EtOAc. After the mixture was stirred for 0.5 h, filtration gave **30a**·HCl (0.18 g, 31%): mp >300 °C; ^{1}H NMR δ 12.59 (s, 1 H), 12.04 (s, 1 H), 9.17 (d, 1 H), 8.04 (s, 1 H), 7.87 (d, 1 H), 7.29 (s, 1 H); MS (FAB) m/z 274 (M + 1). Anal. ($C_{11}H_8N_5O_4$·HCl·H_2O) C, H, Cl, N.

6-(3-Pyridyl)-2,3(1*H*,4*H*)-quinoxalinedione (29b). A solution of **28b** (4.29 g, 15.5 mmol) in 1 N HCl (40 mL) was refluxed for 1 h and evaporated. The residue was hydrogenated at atmospheric pressure using Pd–C (10%, 0.2 g) as catalyst in methanol (30 mL) and concentrated HCl (2 mL). After filtration followed by evaporation, the residue was treated with oxalic acid (1.50 g, 16.7 mmol) in 4 N HCl (48 mL) to give **29b** (4.45 g, 97%): mp >300 °C; ^{1}H NMR δ 12.16 (br s, 2 H), 8.60–9.10 (m, 3 H), 7.26–7.63 (m, 3 H), 6.56 (br s, 1 H); MS (FAB) m/z 240 (M + 1). Anal. ($C_{13}H_9N_3O_2$·HCl·0.2H_2O) C, H, Cl, N.

6-Nitro-7-(3-pyridyl)-2,3(1*H*,4*H*)-quinoxalinedione Semisulfate (30b·1/2H_2SO_4). To an ice-cold solution of **29b** (1.0 g, 3.63 mmol) in concentrated H_2SO_4 (10 mL) was added ^{f}HNO$_3$ (0.17 mL, 3.7 mmol) dropwise. After being stirred for 1 h at room temperature, the solution was poured onto ice (100 g) and the resulting solid was recrystallized from ethanol–H_2O to give **30b**·1/2H_2SO_4 (0.53 g, 44%): mp >300 °C; ^{1}H NMR δ 12.42 (br s, 1 H), 7.97 (s, 1 H), 7.81 (dd, 1 H), 7.12 (s, 1 H), 6.28 (br s, 1 H); MS (FAB) m/z 285 (M + 1). Anal. ($C_{13}H_8N_4O_4$·0.55H_2SO_4) C, H, N, S.

General Method for Preparation of Quinoxaline-2,3-(1*H*,4*H*)-diones. Quinoxaline-2,3(1*H*,4*H*)-diones **45–50** and **52** were prepared by hydrogenation of the appropriate *o*-nitroanilines under atmospheric pressure using Pd–C to give the diamines followed by reaction with oxalic acid in 4 N HCl at reflux temperature to give the corresponding quinoxaline-2,3(1*H*,4*H*)-diones.

4-(1*H*-Imidazol-1-yl)-5-methyl-2-nitroaniline (35a). A mixture of **31a** (9.8 g, 63 mmol) and imidazole (21 g, 308 mmol) was heated at 120 °C for 3 h. To this hot mixture was added ice–water to give compound **32a** as a yellow solid. The solid was hydrogenated under atmospheric pressure with Pd–C (10%, 1.6 g) in methanol (130 mL), concentrated HCl (12 mL), and evaporated after removing the catalyst. The resulting residue was treated with acetic anhydride (7.7 mL, 75.4 mmol) and triethylamine (33 mL, 229 mmol) in $CHCl_3$ (150 mL) at room temperature. After being stirred overnight, the solution was diluted with $CHCl_3$, washed with water, saturated aqueous

$NaHCO_3$, and diluted HCl, dried, and evaporated to give an oil, which was treated with hexane–ether to give solid **33a** (9.8 g, 72%).

To a solution of **33a** (3.3 g, 15.3 mmol) in concentrated H_2SO_4 (12 mL) was added KNO_3 (1.7 g, 16.8 mmol) portionwise at 0–10 °C. After being stirred for 1.5 h at room temperature, the solution was poured onto ice and neutralized with aqueous NaOH. The solution was extracted with $CHCl_3$, dried, and evaporated to give solid **34a**, which was treated with hot (60 °C) 1 N HCl (100 mL) overnight. After neutralization with 1 N NaOH, the solution was extracted with a large amount of $CHCl_3$, dried, and evporated. The residue was recrystallized from the minimum amount of $CHCl_3$ to give **35a** (1.67 g, 49%): mp 190 °C dec; ^{1}H NMR δ 7.84 (s, 1 H), 7.78 (s, 1 H), 7.59 (s, 2 H), 7.07 (s, 1 H), 6.98 (s, 1 H), 2.03 (s, 3 H); MS (EI) *m/z* 218 (M).

5-Fluoro-4-(1*H*-imidazol-1-yl)-2-nitroaniline (35b). A solution of 3,4-difluoronitrobenzene (**31b**) (10 g, 62.9 mmol) and imidazole (20 g, 0.29 mmol) in dry DMF (100 mL) was heated at 80 °C overnight and then evaporated to a small volume. To this residue was added water to give a precipitate, **32b** (11.2 g, 86%).

A solution of **32b** (9.44 g, 45.6 mmol) in EtOH (100 mL) was hydrogenated at atmospheric pressure using Raney Ni (2 g) as catalyst. The suspension was filtered through Celite under N_2, and the filtrate was evaporated to give a residue (8.1 g), which was treated with acetic anhydride (5.7 mL, 55.9 mmol) and triethylamine (10 mL, 69.3 mmol) in $CHCl_3$ (40 mL) at room temperature for 1.5 h. The resulting precipitate was filtered and washed with $CHCl_3$ to give solid **33b** (8.5 g, 84%).

To an ice-cold solution of **33b** (5 g, 22.8 mmol) in concentrated H_2SO_4 (35 mL) was added KNO_3 (2.53 g, 25.0 mmol). The resulting mixture was stirred for 1 h, and then, more KNO_3 (1.23 g, 12.2 mmol) was added at the same temperature. After 2.5 h, the reaction mixture was poured onto ice and neutralized with aqueous NaOH and the mixture was filtered off to give solid **34b** (5 g, 83%). A mixture of this (3 g, 11.4 mmol) in 4 N HCl (30 mL) was refluxed for 2 h and then evaporated to give a solid, which was dissolved in water (50 mL) and neutralized to give a crystalline of **35b** (2.27 g, 88%): mp 195 °C dec; ^{1}H NMR δ 8.18 (d, *J* = 8.3 Hz, 1 H), 7.95 (s, 1 H), 7.80 (s, 2 H), 7.49 (s, 1 H), 7.10 (s, 1 H), 7.00 (d, *J* = 12.7 Hz, 1 H); MS (EI) *m/z* 222 (M).

4-(1*H*-Imidazol-1-yl)-5-(trifluoromethyl)-2-nitroaniline (35c). A solution of 2-fluoro-5-nitrobenzotrifluoride (**31c**) (3.29 g, 15.7 mmol) in acetic acid (20 mL) was hydrogenated under atomspheric pressure with 10% Pd–C as catalyst. The suspension was filtered, and the filtrate was evaporated. To a solution of this residue (2.8 g) in $CHCl_3$ (28 mL) were added acetic anhydride (1.6 mL, 15.7 mmol) and triethylamine (4.9 mL, 34.0 mmol), and the resulting mixture was stirred at room temperature overnight. After dilution with $CHCl_3$, the reaction mixture was washed with 1 N NaOH, 1 N HCl, and water, dried, and evaporated. The residue was recrystallized from EtOAc–hexane to give a crystalline of **37** (2.54 g, 88%).

To a cold (0 °C) ^{f}HNO$_3$ (50 mL) was added **37** (9.4 g, 42.5 mmol) portionwise, and the resulting mixture was stirred for 1 h in an ice bath. The solution was poured into ice–water (500 mL) to give a precipitate (9.85 g), which was recrystallized twice from isopropyl alcohol–water to give **38** (4.67 g, 41%). The mother solutions were evaporated, and the resulting solid was chromatographed (eluant: $CHCl_3$–hexane 1:1–1:0) to give **38** (4.83 g, total 84%) and **39** (0.97 g, 8.6%). **38**: mp 111–112 °C; ^{1}H NMR δ 10.46 (br s, 1 H), 8.23 (d, $J_{H,F}$ = 10.3 Hz, 1 H), 8.01 (d, $J_{H,F}$ = 6.8 Hz, 1 H), 2.08 (s, 3 H). **39**: mp 114–115 °C; ^{1}H NMR δ 10.06 (br s, 1 H), 8.02 (dd, $J_{H,F}$ = 5.3 Hz, $J_{H,H}$ = 9.2 Hz, 1 H), 7.83 (t, $J_{H,F}$ = $J_{H,H}$ = 9.2 Hz, 1 H), 2.08 (s, 3 H).

A solution of **38** (1.5 g, 5.63 mmol) and imidazole (1.9 g, 27.9 mmol) in DMF (7.5 mL) was heated at 140 °C overnight. After cooling to room temperature, the reaction mixture was poured into ice–water to give a solid, which was taken up in 4 N HCl (15 mL) and heated at reflux for 1.5 h. After filtration, the solution was cooled and washed with isopropyl ether. The aqueous layer was evaporated to give **35c** (1.13 g, 65%): mp 185 °C dec; ^{1}H NMR δ 9.50 (s, 1 H), 8.56 (s, 1 H), 8.33 (s, 2 H), 7.95 (s, 1 H), 7.85 (m, 2 H); MS (EI) *m/z* 272 (M).

4,5-Di(1*H*-imidazol-1-yl)-2-nitroaniline (36). A solution of **34b** (1 g, 3.79 mmol) and imidazole (0.65 g, 9.5 mmol) in DMF (10 mL) was heated at 100 °C for 2.5 h. The reaction mixture was evaporated to give a residue, which was dissolved in $CHCl_3$. The organic layer was washed with water, dried, and evaporated to give a solid, which was recrystalized from EtOAc–$CHCl_3$ (mp 211–212 °C). This product (1 g, 3.2 mmol) was refluxed in 2 N HCl (10 mL) for 1.5 h, and then, the solution was evaporated to give **36** (1.0 g, 77%) as its dihydrochloride salt: mp 182–184 °C; ^{1}H NMR δ 9.36 (m, 1 H), 9.31 (m, 1 H), 8.62 (s, 1 H), 8.27 (m, 2 H), 7.71 (m, 4 H), 7.53 (s, 1 H); MS (EI) *m/z* 269 (M – 1).

4-(1*H*-Imidazol-1-yl)-2-nitro-3-(trifluoromethyl)aniline (40). A solution of **39** (1 g, 3.76 mmol) and imidazole (1.3 g, 19.1 mmol) in DMF (5 mL) was heated at 140 °C for 2 h. A large amount of water was added to the solution to give a solid, which was dissolved in 4 N HCl (10 mL) and refluxed for 5 h. After the solution cooled, the resulting crystalline product was collected to afford **40** (0.58 g, 56%): mp 220 °C dec; ^{1}H NMR δ 9.46 (s, 1 H), 8.01 (s, 1 H), 7.87 (s, 1 H), 7.66 (d, *J* = 9 Hz, 1 H), 7.39 (d, *J* = 9 Hz, 1 H), 6.93 (br m, 3 H); MS (EI) *m/z* 272 (M).

4-Cyano-5-(1*H*-imidazol-1-yl)-2-nitroaniline (44a). 2,4-Difluorobenzonitrile (**41a**) (13 g, 93.5 mmol) was added to hot ^{f}HNO$_3$ (60 °C, 65 mL) portionwise, and the solution was stirred for 1 h and cooled. The solution was poured onto ice (600 g) and extracted with $CHCl_3$. The organic layer was washed with saturated aqueous NaCl, dried, and evaporated to give an oil, **42a** (13 g, 75%). To a solution of **42a** (16 g, 87.0 mmol) in ethanol (10 mL) was added 28% ammonia (50 mL), and the reaction mixture was stirred for 0.5 h. The resulting crystalline material was filtered off and washed with water to give **43a** (8.5 g, 54%, mp 199–200 °C). A solution of **43a** (1 g, 5.52 mmol) and imidazole (1.1 g, 16 mmol) in DMF (5 mL) was heated at 100 °C for 1 h and poured into water. The resulting product was collected by filtration to give **44a** (1.26 g, 99%): mp 220–222 °C; ^{1}H NMR δ 8.64 (s, 1 H), 8.22 (2 H), 8.10 (br s, 1 H), 7.61 (br s, 1 H), 7.18 (br s, 1 H), 7.11 (s, 1 H); MS (EI) *m/z* 229 (M).

4-Acetyl-5-(1*H*-imidazol-1-yl)-2-nitroaniline (44b): 22% from **41b** by three steps; ^{1}H NMR δ 8.52 (s, 1 H), 8.03 (br s, 1 H), 7.84 (s, 1 H), 7.37 (t, 1 H), 7.08 (s, 1 H), 6.98 (s, 1 H), 2.18 (s, 3 H); MS *m/z* 247 (M + 1).

7-Cyano-6-(1*H*-imidazol-1-yl)-2,3(1*H*,4*H*)-quinoxalinedione Hydrochloride (45·HCl): 37% from **44a**; mp >300 °C; ^{1}H NMR δ 12.69 (s, 1 H), 12.48 (s, 1 H), 9.54 (s, 1 H), 8.14 (s, 1 H), 7.90 (s, 1 H), 7.73 (s, 1 H), 7.52 (s, 1 H); MS (FAB) *m/z* 254 (M + 1). Anal. ($C_{12}H_7N_5O_2$·HCl) C, H, Cl, N.

6-(1*H*-Imidazol-1-yl)-7-(trifluoromethyl)-2,3(1*H*,4*H*)-quinoxalinedione Hydrochloride (46·HCl): 89% from **35c**; mp >300 °C; ^{1}H NMR δ 12.65 (s, 1 H), 12.48 (s, 1 H), 9.48 (s, 1 H), 8.02 (s, 1 H), 7.88 (s, 1 H), 7.73 (s, 1 H), 7.52 (s, 1 H); MS (FAB) *m/z* 297 (M + 1). Anal. ($C_{12}H_7F_3N_4O_2$·HCl·H_2O) C, H, Cl, F, N.

7-Acetyl-6-(1*H*-imidazol-1-yl)-2,3(1*H*,4*H*)-quinoxalinedione Hydrochloride (47·HCl): 23% from **44b**; mp >300 °C; ^{1}H NMR δ 12.56 (s, 1 H), 12.34 (s, 1 H), 9.39 (s, 1 H), 7.92–7.84 (m, 3 H), 7.34 (s, 1 H), 2.45 (d, 3 H); MS (FAB) *m/z* 271 (M + 1). Anal. ($C_{13}H_{10}F_3N_4O_3$·HCl·1.7H_2O) C, H, Cl, N.

7-Fluoro-6-(1*H*-imidazol-1-yl)-2,3(1*H*,4*H*)-quinoxalinedione Hydrochloride (48·HCl): 64% from **35b**; mp >300 °C; ^{1}H NMR δ 12.37 (br s, 2 H), 9.53 (d, *J* = 1.3 Hz, 1 H), 8.07 (d, *J* = 1.3 Hz, 1 H), 7.90 (t, 1 H), 7.46 (d, *J* = 7 Hz, 1 H), 7.31 (d, *J* = 11 Hz, 1 H); MS *m/z* 246 (M). Anal. ($C_{11}H_7FN_4O_2$·HCl·1.05H_2O) C, H, Cl, N; N: calcd, 3.38; found, 3.84.

6-(1*H*-Imidazol-1-yl)-7-methyl-2,3(1*H*,4*H*)-quinoxalinedione Hydrochloride (49·HCl): 76% from **35a**; mp >300 °C; ^{1}H NMR δ 12.17 (br s, 2 H), 9.37 (t, *J* = 1.3 Hz, 1 H), 7.98 (t, *J* = 1.3 Hz, 1 H), 7.89 (t, 1 H), 7.20 (s, 1 H), 7.16 (s, 1 H), 2.11 (s, 3 H); MS *m/z* 243 (M + 1). Anal. ($C_{12}H_{10}N_4O_2$·HCl) C, H, Cl, N.

6,7-Di(1*H*-imidazol-1-yl)-2,3(1*H*,4*H*)-quinoxalinedione Dihydrochloride (50·2HCl): 24% from **36**; mp >300 °C; ^{1}H NMR δ 12.66 (br s, 2 H), 9.32 (br m, 2 H), 7.73 (br m, 4 H), 7.58 (br m, 2 H); MS (FAB) *m/z* 295 (M + 1). Anal. ($C_{14}H_{10}N_6O_2$·2.2HCl·2.27H_2O) C, H, Cl, N.

6-Amino-7-(1*H*-imidazol-1-yl)-2,3(1*H*,4*H*)-quinoxalinedione Hydrochloride (51·2HCl). A mixture of **11** (1 g, 3.23 mmol) in 4 N HCl (20 mL) was hydrogenated at atmospheric pressure using Pd–C (10%, 180 mg). The suspension was filtered through Celite under N_2, and the filtrate was evaporated to dryness to give crystalline **51** as the HCl salt (500 mg, 46%): mp > 300 °C;

^{1}H NMR δ 12.15 (s, 1 H), 12.06 (s, 1 H), 9.48 (s, 1 H), 7.93 (s, 1 H), 6.92 (s, 1 H), 7.90 (s, 1 H), 7.12 (s, 1 H); MS (FAB) *m/z* 244 (M + 1). Anal. ($C_{11}H_9N_5O_2 \cdot 2HCl \cdot H_2O$) C, H, Cl, N.

6-(1*H*-Imidazol-1-yl)-5-(trifluoromethyl)-2,3(1*H*,4*H*)-quinoxalinedione (52): 25% from **40**; mp >300 °C; ^{1}H NMR δ 12.33 (br s, 2 H), 7.80 (s, 1 H), 7.47 (d, *J* = 8.5 Hz, 1 H), 7.37 (s, 1 H), 7.25 (d, *J* = 8.5 Hz, 1 H), 7.08 (s, 1 H); MS (FAB) *m/z* 297 (M + 1). Anal. ($C_{12}H_7F_3N_4O_2 \cdot 0.2H_2O$) C, H, F, N.

Biology. Radiobinding Assay. Inhibition of the specific binding of [^{3}H]AMPA, [^{3}H]KA, NMDA-sensitive [^{3}H]Glu, and strychnine-insensitive [^{3}H]Gly to brain membranes in vitro was evaluated using standard procedures.

The binding of [^{3}H]AMPA was conducted with crude membranes of rat whole brain in the presence of 100 mM KSCN as described by Honore et al.[19] [^{3}H]KA binding was performed using crude membranes from rat cortex.[24] [^{3}H]Glu and [^{3}H]Gly bindings were examined using Triton X-100-treated membranes of whole brain except cerebellum.[25,26] Final ligand concentrations were as follows: [^{3}H]AMPA, 43 nM; [^{3}H]KA, 4 nM; [^{3}H]Glu, 10 nM; and [^{3}H]Gly, 35 nM.

K_i values were determined using the Cheng–Prusoff relationship and IC_{50} values were determined from logit–log analysis.

Glutamate Receptor Agonist Neurotoxicity. Male Wistar rats were anesthetized with pentobarbitone (50 mg/kg, ip) and placed in a David Kopf small animal stereotaxic apparatus. Two microliters of solution was injected unilaterally into the striatum (I, 8.7; L, 3.0; and V, 5.5). The injected drug was dissolved in phosphate-buffered saline (pH 7.4). For coinjection experiments, the drug (both singly or as coinjection) was dissolved in aqueous NaOH (pH 9–10). All injections were carried out over 2 min with the injection cannulae left in place for a further 5 min. Seven days later, the animals were killed by decapitation and their brains were removed and placed on ice. Coronal sections, 2.5 mm in thickness, were made with a razor blade. The frontal poles were removed, and the second coronal cut passed through the anterior commissure. Both striata were dissected and stored frozen at −80 °C until assay.

Enzyme Assay. Tissues were homogenized in 25 mM phosphate buffer (pH 7.4). Choline acetyltransferase was assayed at pH 7.4 in a total volume of 200 μL containing final concentrations (mM) of sodium phosphate (100), EDTA (20), NaCl (300), choline (10), acetyl-CoA (0.4), and physostigmine (0.2). Following 10 min at 37 °C, 50 μL of 1 M perchloric acid was added to the incubation mixtures. Formed acetylcholine was separated and measured by HPLC-ECD. The HPLC-ECD system included a pump (Hitachi, L6210), an autosampler (BAS Japan, CAM 200/220), a guard and chromatographic columns (BAS Japan, 5- × 4-mm and 60- × 4-mm), acetylcholinesterase and choline oxidase fixed on an immobilized enzyme reactor column (BSA Japan, 5- × 4-mm), and ECD (BAS Japan, LC-4C). The mobile phase consisted of 50 mM Na_2HPO_4, 1 mM EDTA, and 0.5 mM sodium octanesulfonate. The flow rate of the mobile phase was 0.8 mL/min. The applied potential at the working electrode was 450 mV vs Ag/AgCl.

Audiogenic Seizures in DBA/2 Mice. Test compounds were given ip or icv to groups of 10 male DBA/2 mice (21–28 days old; weight 10–12 g) per dose level 15 min prior to challenge with auditory stimulation (12 kHz at 120 dB).[29] Seziure response was assessed on the following scale: 0 = no response, 1 = wild running, 1 = clonus, 1 = tonic, 1 = respiratory arrest, maximum score = 4, minimum score = 0.

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