Expedited Articles

Synthesis of 1,4,7,8,9,10-Hexahydro-9-methyl-6-nitropyrido[3,4-*f*]-quinoxaline-2,3-dione and Related Quinoxalinediones: Characterization of α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic Acid (and *N*-Methyl-D-aspartate) Receptor and Anticonvulsant Activity[1]

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Four related series of substituted quinoxalinediones containing angular fused-piperidine rings have been synthesized as α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor antagonists with potential as neuroprotective agents, primarily for acute therapy immediately following a stroke.[2] The compounds were tested for their affinity to the AMPA, kainate, and strychnine-insensitive glycine receptor sites. In AMPA binding, the most potent compound was **27a** (PNQX, IC_{50} = 63 nM), with affinity comparable to the literature standard **1** (NBQX, IC_{50} = 52 nM). Other 6-nitro analogs from the 9-aza series had comparable affinity at the AMPA receptor, as did 6-nitro-8-aza derivatives such as **13a** (iPNQX, IC_{50} = 290 nM). The receptor binding profile of **27a** differed from that of **1** in that **27a** possessed significant affinity at the glycine site of the *N*-methyl-D-aspartate (NMDA) receptor, whereas **1** was essentially inactive. Three compounds, **26c**, **26d**, and **26e**, demonstrated moderate selectivity for kainate relative to AMPA receptors. Selected analogs reported herein as well as in the literature were superimposed to generate an AMPA pharmacophore model, and 6-substituted compounds from the PNQX and iPNQX series were combined and analyzed via quantitative structure–activity relationship techniques. Compounds with high affinity at non-NMDA receptors were further characterized in functional assays in neuronal cell culture and in a cortical wedge preparation. Both **1** and **27a** showed comparable effectiveness in an AMPA- and kainate-induced excitoxicity assay. Both inhibited AMPA-induced depolarizations in the cortical wedge. However, **27a** also inhibited spontaneous epileptiform discharges in the cortical wedge (reversed by glycine), while **1** was ineffective. The combination of AMPA and NMDA antagonist activity may contribute to the 30-fold difference in potency between **27a** and **1** in the maximal electroshock convulsant assay in mice. The significant *in vivo* potency of **27a** suggests that it has potential clinical utility.[2]

Introduction

Under pathological conditions, excitatory amino acid (EAA) neurotransmitters acting at both *N*-methyl-D-aspartate (NMDA) and non-NMDA glutamate receptors, including α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) and kainate receptors, can cause excess excitation that initiates neuronal cell death. By interrupting this excitotoxic cascade, EAA antagonists may prevent the neuronal cell death that is characteristic of many neurological disorders.[3–5] Because glutamate excitotoxicity may be a result of action at more than one glutamate receptor subtype, inhibition of multiple subtypes may be required for complete protection against excitotoxic injury. Three general types of ionotropic or "channel-containing" glutamate receptors have been identified via molecular cloning of receptor subtypes and can be differentiated by their binding preferences for the synthetic agonists NMDA, AMPA, and kainate. All three subclasses of glutamate receptors are composed of multiple subunits (GluR 1–4 for AMPA receptors, Glu R 5–7 and KA 1–2 for kainate receptors, and NR1A combined with NR2 A–D for NMDA receptors) in an undefined stoichiometry.[6] AMPA receptor antagonists as a class have demonstrated good neuroprotective activity; examples include LY 215490 (**2**)[7,8] and YM90K (**3**).[9] 2,3-Dihydroxy-6-nitro-7-sulfamoylbenzo[*f*]quinoxaline (**1**, NBQX) has emerged as a reference standard for this class of antagonists.[10] It has neuroprotective activity in models of focal[11–13] and global[1,14–16] cerebral ischemia, as well as in transient spinal cord ischemia.[17] In addition to ischemia, studies support its use as an anticonvulsant in epilepsy,[18–21] as a neuroprotectant in spinal cord trauma,[22] and potentially as a treatment for neurodegenerative disorders such as Parkinsonism[23] and amyotrophic lateral sclerosis (ALS).[24,25] However, the clinical utility of **1**

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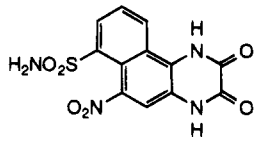
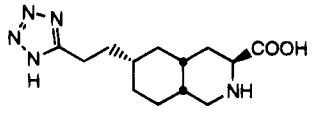
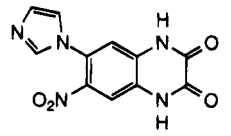
may be limited by undesirable physical properties and pharmacokinetics and by potential nephrotoxicity.[26]



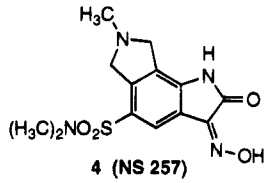
1 (NBQX)



2 (LY 215490)



3 (YM90K)



4 (NS 257)

NMDA receptor antagonists also have neuroprotective properties, and might be useful for acute cerebral ischemia, such as in stroke[27] or head trauma,[28] or in chronic neurodegenerative disorders such as Parkinson's disease,[29,30] human immunodeficiency virus (HIV)-related neuronal injury,[31,32] ALS,[33] Alzheimer's disease,[30,34] and Huntington's disease.[35,36] Recent studies have shown that combinations of NMDA and non-NMDA receptor antagonists act synergistically in models of focal and global ischemia,[37] in seizure disorders[38,39] and in protection of neuronal degeneration in the retina.[40] Thus a compound with both NMDA and AMPA antagonist properties might have neuroprotective activity exceeding that of a selective antagonist.

The purpose of this study was to prepare potent AMPA receptor antagonists and to identify the most viable candidates for pharmacological characterization *in vivo*. Compounds with high intrinsic activity at the AMPA receptor were examined for activity at the glycine site of the NMDA receptor, since it has been established that there is considerable similarity in structural requirements for binding at the AMPA and glycine recognition sites.[41,42] The ability of compounds to penetrate the central nervous system (CNS) was assessed by blockade of maximal electroshock seizures (MES) in mice; this assay also served as a surrogate measure of neuroprotective potential. Previously, we reported NS 257 (**4**) to be a modestly potent, selective AMPA receptor antagonist with improved aqueous solubility relative to **1**.[43] We hypothesized that combining the fused cyclic amine structure of **4** with the quinoxalinedione ring of **1** might produce potent AMPA antagonists with the increased aqueous solubility necessary for formulation of infusion solutions needed for acute conditions such as stroke. To test this idea, several series of analogs that feature a "nitrogen walk" around a fused piperidine ring have been prepared. Identification and superposition of functional groups found to be required for significant AMPA affinity has led to an AMPA pharmacophore model, the generation of which will be described. In addition, the rational design and preparation of a number of 5-substituted analogs within the fused-piperidine quinoxalinedione (PNQX and iPNQX[44]) series has allowed a quantitative assessment of the contribution of this group to AMPA affinity to be carried out using QSAR techniques.

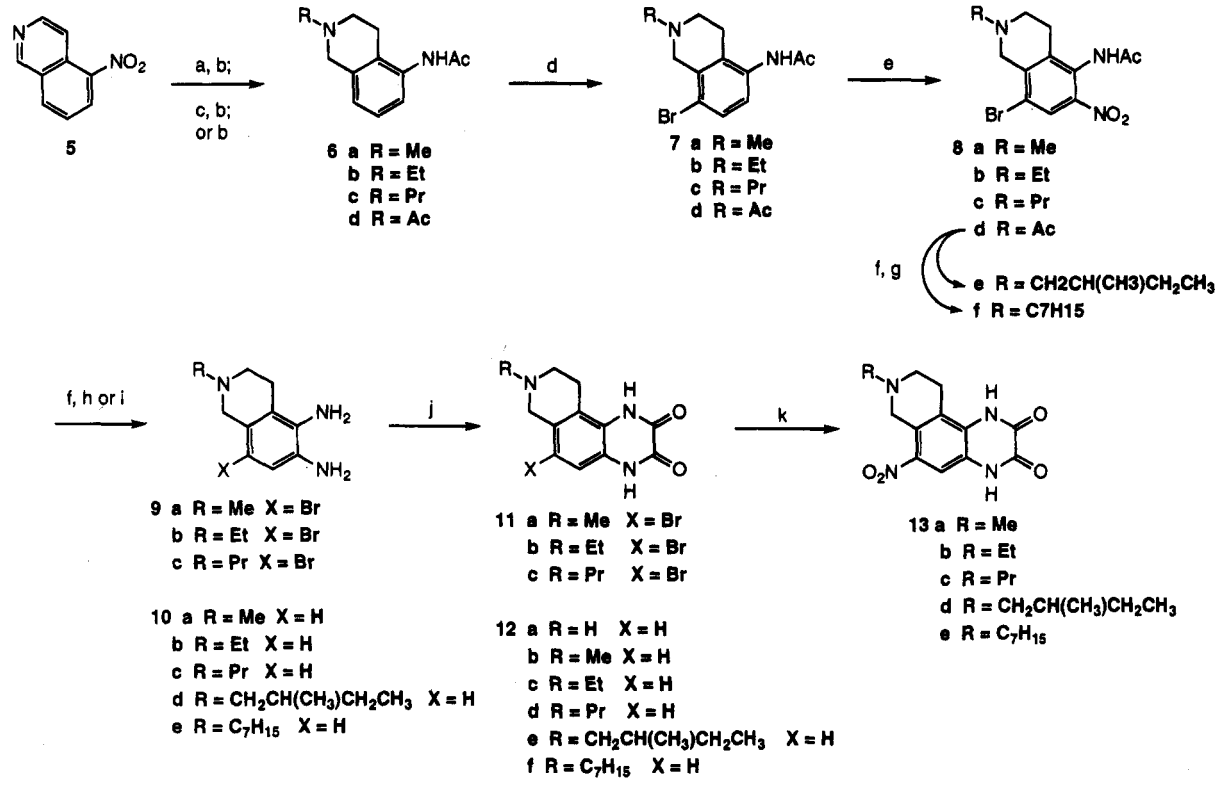
Chemistry

The compounds described in this study were originally designed as a chimera of the quinoxalinedione moiety of **1** and the fused azacycloalkyl moiety of **4**, in an attempt to maintain high AMPA affinity while improving aqueous solubility. While the quinoxalinedione framework was retained, synthetic strategies were needed to allow selective positioning of a substituted nitrogen atom around the fused piperidine ring. From previous work on quinoxalinediones[45] and other templates,[46] aromatic substitution was expected to have a large impact on potency at the AMPA receptor. In addition, the effect on physical properties and *in vivo* activity could be explored via substitution of the piperidine ring nitrogen. Such compounds would also prove useful in mapping the steric requirements of the AMPA receptor in this region.

In Scheme 1, the strategy for synthesis of the 8-aza analogs (iPNQX series[44]) is shown. Starting with 5-nitroisoquinoline **5**, the ring nitrogen can be alkylated to form the isoquinolinium ion using standard conditions, followed by catalytic hydrogenation using platinum oxide reduces both the nitro functionality and the isoquinoline ring to the tetrahydroisoquinoline. The presence of acetic anhydride allows the aniline to be trapped as the acetamides **6a–c**. Alternatively, **5** can be hydrogenated directly with platinum oxide in acetic acid and trapped with acetic anhydride as the bisacetamide **6d**. Bromination of **6a–d** proceeds smoothly with bromine in a mixture of trifluoroacetic and acetic acids to give the 8-bromo derivatives **7**. Subsequent nitration with fuming nitric acid in trifluoroacetic acid provides fully elaborated tetrahydroisoquinolines **8a–d** with the desired regiochemistry. Hydrolysis of the functionalized bisacetamide **8d**, followed by reductive amination with an aldehyde, gave **8e** and **8f**, which permits a highly divergent synthetic strategy. The nitro group can be selectively reduced using deactivated Raney nickel as catalyst to give the bromo diamines **9**, or both the bromo and nitro groups can be simultaneously reduced using palladium as catalyst to give the diamines **10**. Condensation with oxalic acid in aqueous refluxing 3 N HCl provided the quinoxalinediones **11** and **12**. Nitration of **12** proceeds regioselectively at C-6 to give the quinoxalinediones **13**.

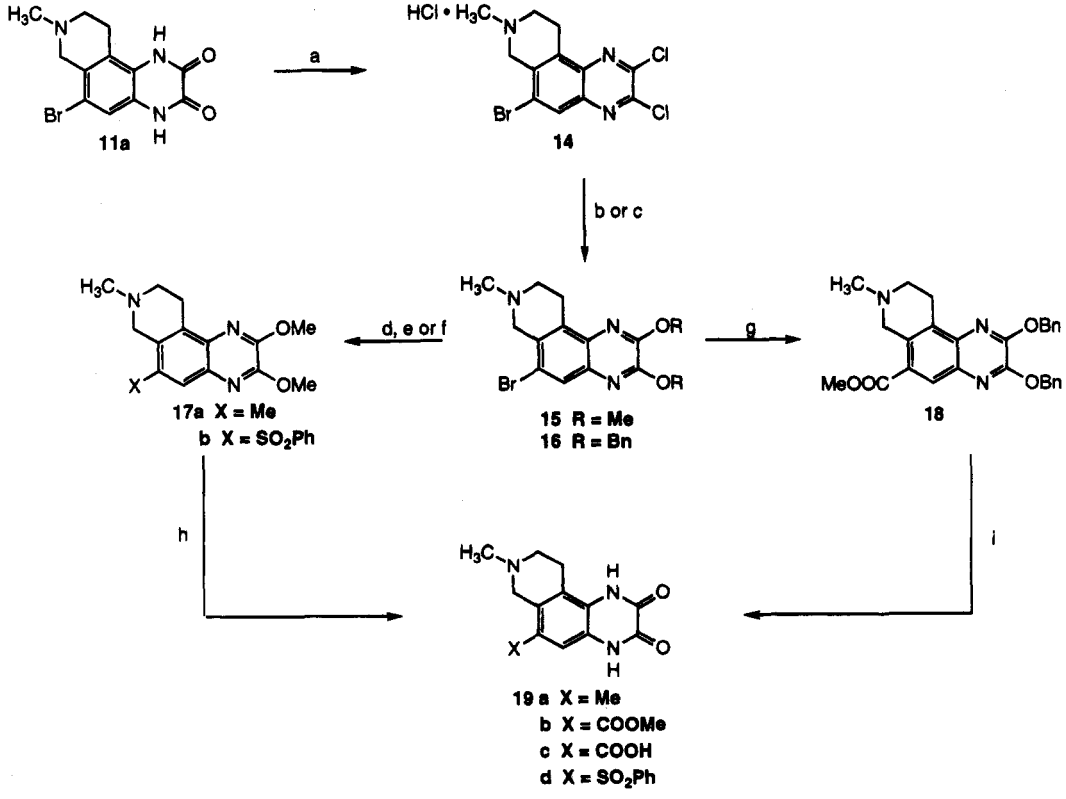
To increase the versatility of this chemistry, methods were developed that allowed alternatives to electrophilic aromatic substitution for elaboration of C-6 substituent. From the 6-bromo derivative **11a**, the bis(imino chloride) **14** was prepared by heating with thionyl chloride in DMF (Scheme 2). Displacement of the chloride with either sodium methoxide or sodium benzyl oxide gave the bis(imino ethers) **15** and **16**. Lithiation of the aryl bromide, followed by alkylation with methyl iodide or benzenesulfonyl fluoride, for example, gave the 6-methyl **17a** and the phenyl sulfone **17b**. Hydrolysis in refluxing 3 N HCl gave the final quinoxalinediones **19a** and **19d** in good overall yield. The bis(benzyl imino ether) **16** can undergo transmetallation with palladium(0) or palladium(II), followed by any number of insertion reactions, as exemplified by preparation of the methyl ester **18**. Hydrogenation of the protecting group gives cleanly the desired quinoxalinedione **19b**. We looked at other bis(imino ethers) (allyl and trimethylsilylethyl) that form in good yield and can be readily removed. The

Scheme 1. Synthetic Routes to iPNQX Series[a]



[a] (a) $CH_3OSO_2CF_3$, CH_2Cl_2; (b) PtO_2, H_2, AcOH, Ac_2O; (c) RX, EtOH, reflux; (d) Br_2, CF_3COOH, AcOH; (e) CF_3COOH, fuming $HONO_2$; (f) 6 N HCl, reflux (or 5:2 H_2SO_4:H_2O); (g) RCHO, $NaCNBH_3$, MeOH, H_2O; (h) H_2, RaNi, THF; (i) H_2, 20% Pd/C, MeOH; (j) $(COOH)_2$ dihydrate, 3 N HCl, reflux; (k) H_2SO_4, $KONO_2$.

Scheme 2. Elaboration of C-6 Aromatic Substitution[a]

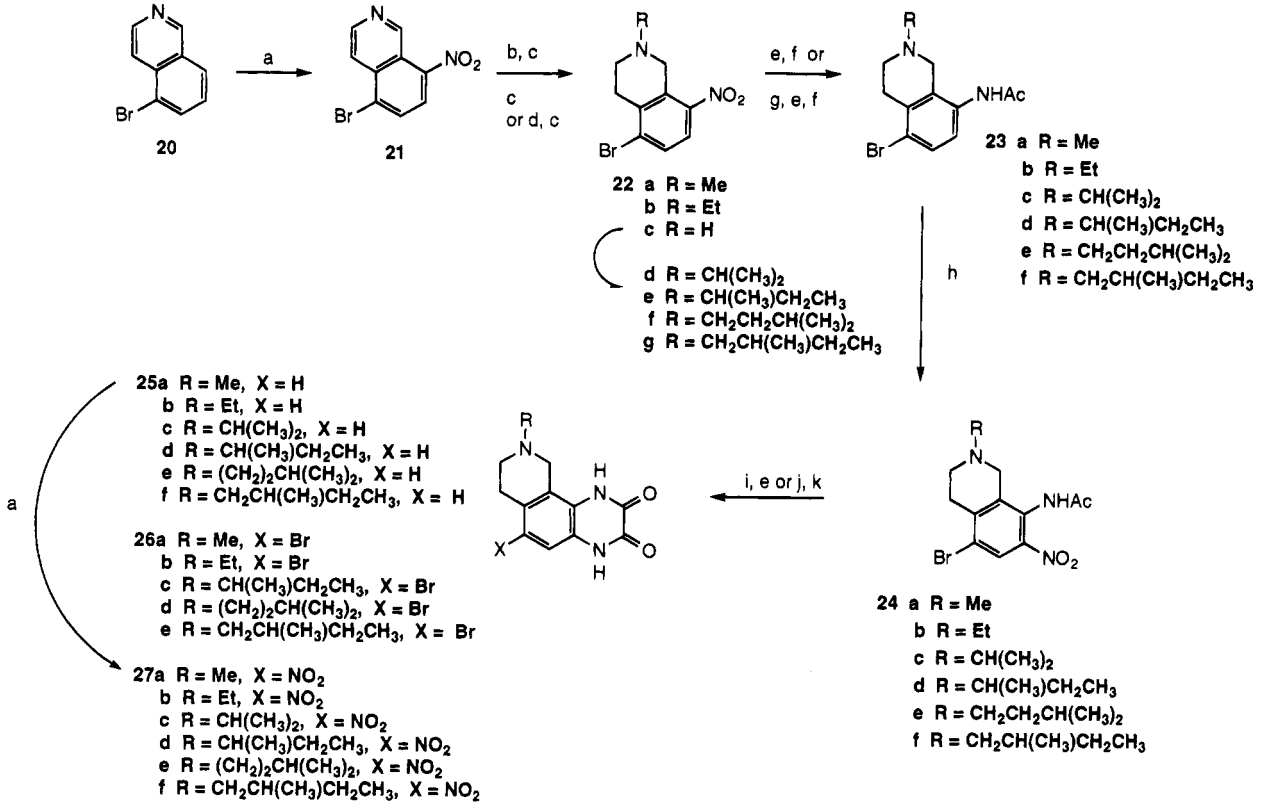


[a] (a) $SOCl_2$, DMF, reflux; (b) MeONa, MeOH; (c) $PHCH_2ONa$, $PHCH_2OH$, THF; (d) *s*-BuLi, THF, −78 °C; (e) MeI; (f) $PhSO_2F$; (g) CO(g), $Pd(Ph_3)_4$, CH_3CN (or toluene), Et_3N, MeOH; (h) 3 N HCl, reflux; (i) H_2, 20% Pd/C, AcOH.

bis[(trimethylsilyl)ethyl imino ether], for example, is prepared from the bis(imino chloride) with (trimethylsilyl)ethanol and sodium hydride, and can be deprotected quantitatively with tetrabutylammonium fluoride to reestablish the quinoxalinedione.

The strategy for synthesis of the 9-aza analogs (PNQX

Scheme 3. Synthetic Routes to PNQX Series[a]



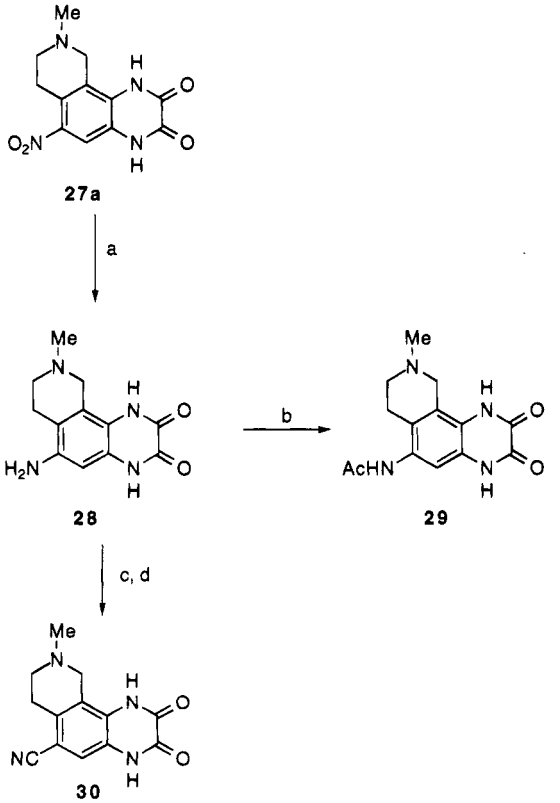
[a] (a) H_2SO_4, $KONO_2$; (b) CF_3SO_3Me, $CHCl_3$; (c) $NaCNBH_3$, HCOOH (or AcOH); (d) $MeSO_3H$, CH_2Cl_2; (e) H_2, RaNi; (f) Ac_2O; (g) R'COR'', $NaCNBH_3$; (h) CF_3COOH, $HONO_2$; (i) 5:2 H_2SO_4:H_2O; (j) H_2, 20% Pd/C; (k) $(COOH)_2$ dihydrate, 3 N HCl, reflux.

series[44]) is shown in Scheme 3. Nitration of 5-bromoisoquinoline **20**[47] with potassium nitrate in concentrated sulfuric acid goes predominantly to C-8. Alkylation of the isoquinoline with methyl triflate, followed by reduction with sodium cyanoborohydride, gave the *N*-methyltetrahydroisoquinoline **22a**. Alternatively, reduction of the isoquinoline with sodium cyanoborohydride in either acetic acid or formic acid provides selectively the *N*-ethyl analog **22b** or the unsubstituted analog **22c**.[48] Selective reduction of the 8-nitro group is accomplished with deactivated Raney nickel, and the amine is trapped with acetic anhydride to give the acetamide **23**. Strategies similar to those used previously provided the 6-H, 6-Br, and 6-NO_2 quinoxalinedione analogs **25**–**27**. The unsubstituted tetrahydroisoquinoline **22c** provides a versatile platform for a range of substitutions. Reductive amination with an aldehyde (or ketone) and sodium cyanoborohydride provides selectively alkylated intermediates **23** that can be taken to the appropriate quinoxalinedione. Notable for its AMPA receptor activity and *in vivo* profile is **27a** (PNQX[45]).

The nitro group of **27a** can be hydrogenated to give the 6-amino analog **28**. Acetylation under standard conditions gave the 6-acetamido **29**. Diazotization of **28**, followed by displacement with potassium tetracyano nickel gave the nitrile **30** (Scheme 4).[49]

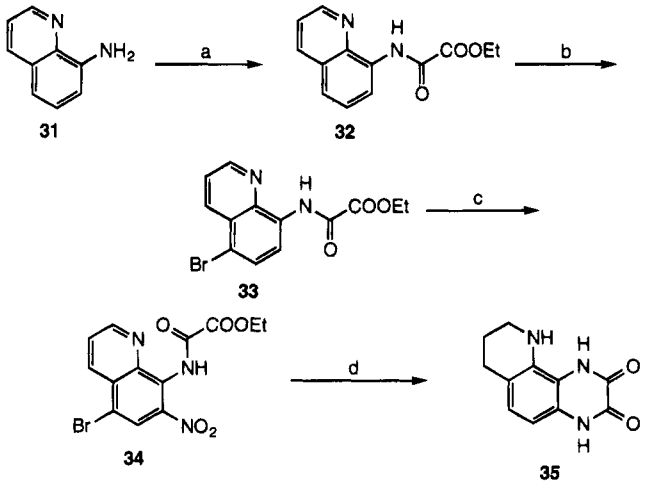
For preparation of the first 10-aza series, 8-aminoquinoline was condensed with ethyl oxalyl chloride, brominated, and then nitrated to give the key intermediate **34**. Hydrogenation in acetic acid with 20% palladium on carbon simultaneously reduced the quinoline ring, removed the aromatic bromine, and reduced the nitro functionality with concomitant ring closure to the quinoxalinedione **35** (Scheme 5). All attempts to selectively nitrate **35** at C-6, with or without protection of N-10, failed. Presumably, the amine changes the electronics of the aromatic ring such that C-6 is no

Scheme 4. Elaboration of C-6 Aromatic Substitution[a]



[a] (a) H_2, 5% Pd/C, AcOH; (b) AcOH, Ac_2O; (c) H_2SO_4, $NaNO_2$; (d) $K_2Ni(CN)_4$, 0–100 °C.

Scheme 5. Synthetic Routes to Quinoline Series A[a]



[a] (a) EtO_2CCOCl, Et_3N, $CHCl_3$; (b) Br_2, AcOH; (c) CF_3COOH, fuming $HONO_2$, 80 °C; (d) H_2, 20% Pd/C, AcOH.

longer preferred. Several attempts at nitration gave a mixture of products.

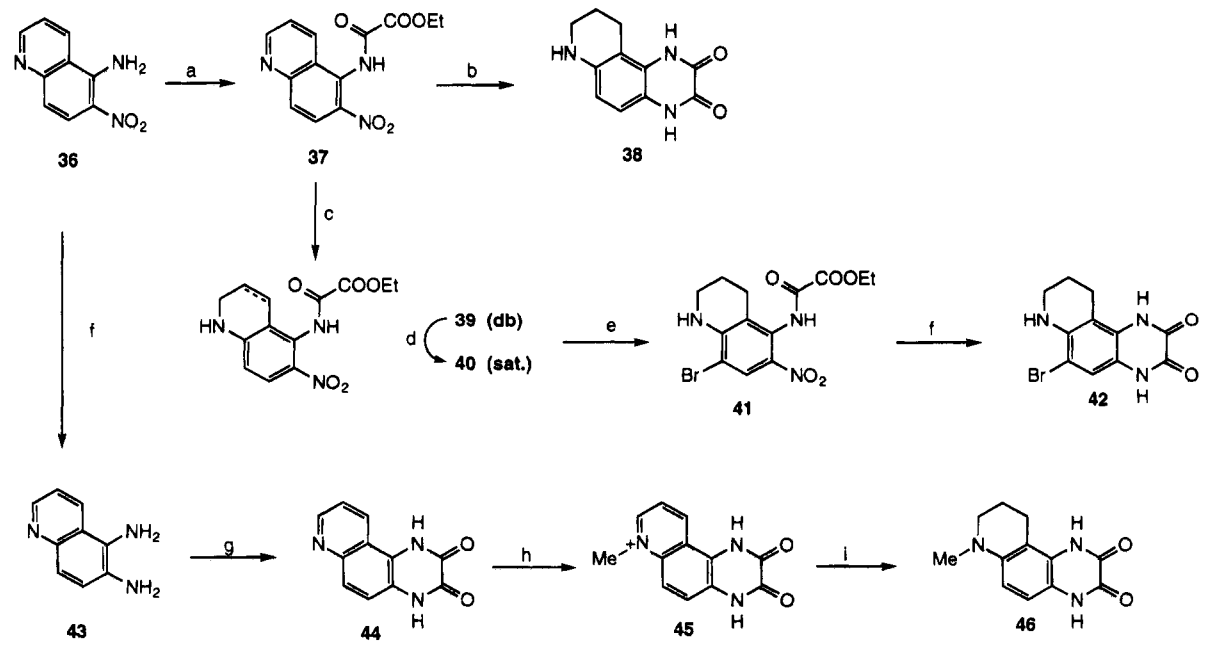
For preparation of the 7-aza series of quinoxalinediones, several strategies were employed. Initially, 5-amino-6-nitroquinoline **36** was condensed with ethyl oxalyl chloride and then hydrogenated over platinum oxide in acetic acid to give the unsubstituted parent 7-azaquinoxalinedione **38**. Sequential reduction of **37** with sodium cyanoborohydride in acetic acid and then with triphenylphosphine and rhodium trichloride under hydrogen (54 psi) in a Parr apparatus gave the tetrahydroisoquinoline while retaining the nitro functionality. Aromatic bromination followed by selective reduction of the nitro group with Raney nickel gave the ring closure onto the oxalate to the quinoxalinedione **42** (Scheme 6). Alternatively, the diaminoquinoline **43** can be closed to the pyridoquinoxalinedione **44** and methylated and the pyrido system reduced to give the 7-methyl derivative **46** (Scheme 6).

Finally, hydrogenation of 5-nitroquinoline in a mixture of acetic acid and acetic anhydride using 5% palladium on carbon as catalyst gave the monoacetylated tetrahydroisoquinoline **48**. To enable directed nitration at the ortho position, N-1 was protected as the butyramide to provide steric interference to inhibit para nitration. Hydrolysis and then acylation with ethyl oxalyl chloride provided **52**, which was converted to the desired quinoxalinedione **53** by reductive ring closure (Scheme 7).

AMPA Pharmacophore Model. In concert with early syntheses in this area, a molecular modeling study was initiated in order to rationalize the observed SAR and aid in the design of novel analogs. To date, there have been relatively few reports on AMPA pharmacophore modeling. Publications generally have been of a preliminary nature, involving the particular ligands under study at the time.[50,51] Our goal was to derive a more comprehensive model, encompassing a number of different series.

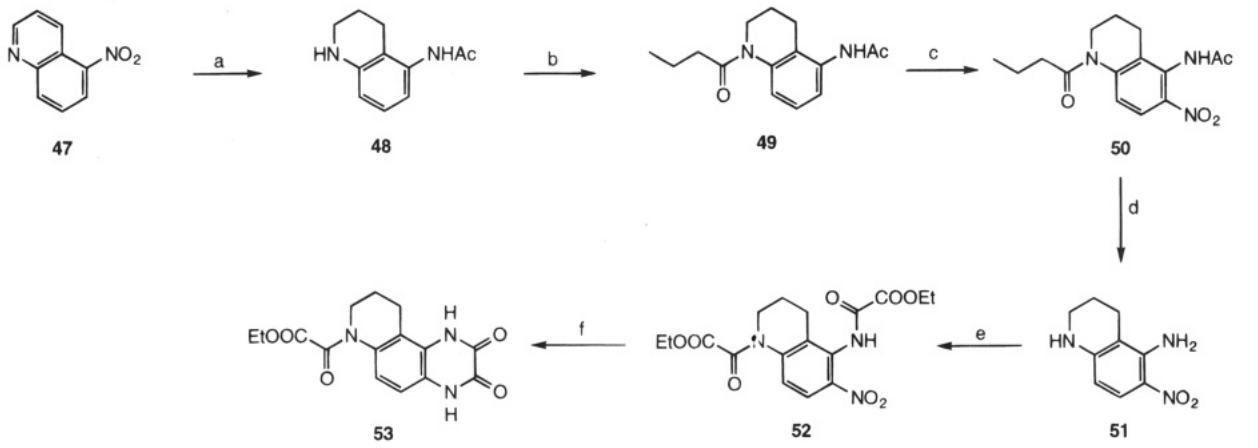
Given the overall similarity in structures and SAR between NMDA glycine-site and AMPA antagonists,[52] the initial modeling was patterned after published glycine pharmacophore models[52-55] (Figure 1), under the assumption that the two receptor sites possessed a substantial degree of similarity. Both glycine and AMPA antagonists possess a critical NH, flanked by a diketo (or keto mimic) functionality thought to form a Coulombic interaction with a positive site on the receptor. Both glycine and AMPA receptors can tolarate substantial bulk to the upper right as shown in Figure 1, and both prefer an electron-withdrawing group at X (Table 3). Differences begin to be seen when bulky groups such as substituted angular fused rings are added to the upper left, to be discussed in more detail below. Many of the most selective compounds for AMPA (YM90K,[8] **11b-e**, **12b**, **13d**; Table 3, series I) possess bulk in this area. In contrast, when the bulk is added that points directly up in Figure 1 ("due north"),

Scheme 6. Synthetic Routes to 7-Aza Series[a]



[a] (a) EtO_2CCOCl, Et_3N, DMF; (b) H_2, PtO_2, AcOH; (c) $NaCNBH_3$, AcOH; (d) H_2, $RhCl_3$, Ph_3P, THF; (e) *N*-bromosuccinimide, DMF; (f) H_2, RaNi, THF; (g) $(COOH)_2$ dihydrate, 3 N HCl, reflux; (h) $(CH_3O)_2SO_2$, DMF, 120 °C; (i) H_2, PtO_2, MeOH.

Scheme 7. Synthetic Routes to 7-Aza Series[a]



[a] (a) H_2, 5% Pd/C, AcOH, Ac_2O; (b) $((C_3H_7)CO)_2O$, Et_3N, CH_2Cl_2, reflux; (c) AcOH, fuming $HONO_2$; (d) 2 N HCl, reflux; (e) EtO_2CCOCl, Et_3N, THF; (f) H_2, 5% Pd/C, EtOH.

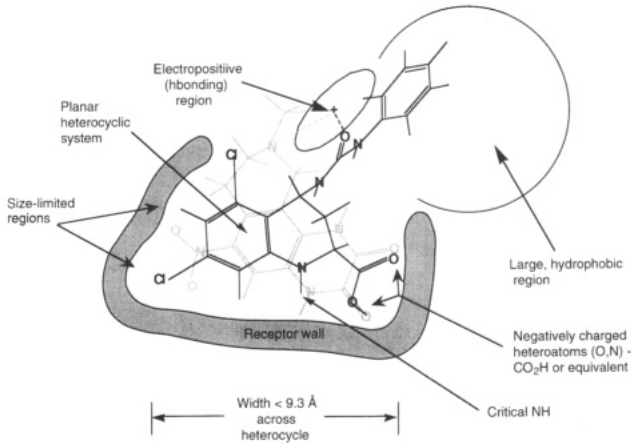


Figure 1. Fits of, in dark lines, *trans*-2-carboxy-5,7-dichlorotetrahydroquinoline-4-phenylurea (L689560; IC_{50} versus [^{3}H]-glycine = 7.8 nM), and in light lines, **27a** (PNQX; IC_{50} vs [3H]glycine = 370 nM), to a pharmacophore model of the glycine site of the NMDA receptor complex.[52] Note the distance requirement for this receptor. The corresponding distance across **1** (NBQX; IC_{50} vs [^{3}H]glycine of >100 μM) is 10.6 Å.

generally nonselective compounds result (**26a–c**, **27a–f**, **30**; Table 3, series II). Differences are accentuated at the 6- and 7-positions (to the left in Figure 1), where either a size-limited pocket exists in the glycine receptor that is just big enough to accommodate a Br or NO_2 (Cl optimal) or there is an overall east to west width requirement by the glycine receptor of <9.3 Å. Thus, NBQX, a potent AMPA binder with relatively large NO_2 and SO_2NH_2 groups in this region (and an overall width of 10.6 Å), is devoid of significant binding at the glycine site. Given these differences, the development of a separate AMPA pharmacophore model seemed justified.

To this end, selected antagonists (Figure 2) were constructed, minimized, and constrained to superimpose the endpoints of 2.8 Å tensors added to the required NH nitrogen atom and one NO_2 oxygen. Included in the superposition were ring centroids as well as both keto oxygens (or keto equivalent nitrogens). See the Experimental Section for details. The tensors were added to simulate interactions with putative receptor site atoms. Overlays of the structures from Figure 2 appear in Figure 3; a schematic of the model is shown in Figure 4. The model entails a presumed Coulombic interaction at A, a critical hydrogen bonding interaction at B, a strong hydrogen bond accepting or Coulombic interaction at C, and a generally coplanar cyclic structure across the heterocyclic rings. A specific hydrogen bonding interaction is not proposed at D; rather, this interaction may be a nonspecific electrostatic interaction with enzyme moieties above and/or below the plane of the ring (ring stacking, or an edge–face interaction with a positive amino acid such as an arginine or lysine). Out of plane volume constraints appear at D and E, while both in- and out-of-plane volume constraints are apparent at F. Use of the model, in concert with the QSAR analysis (*vide infra*), has allowed us to direct synthetic effort toward specic regions in the molecule while avoiding other areas. Results from these efforts will be detailed in additional reports from our laboratories.

QSAR Analysis. Since one of the aims of the present study was an examination of the effect of substituent variation at the 6-position on AMPA affinity, a set of substituents was rationally selected to provide a systematic variation in lipophilic, electronic, steric, and hydrogen-bonding properties.[59] To determine which of these properties might be influencing AMPA affinity, a set of 15 *N*-methyl PNQX and iPNQX analogs (Table 1) containing variations at the 6-position were combined and anaylzed using QSAR techniques.[60] The logarithm of $1/IC_{50}$ versus AMPA was used as the measure of potency; values ranged from 4.0 to 7.0 with a standard error of replicate determinations of ±0.21. Inactive analogs (**19f** and **29**, IC_{50} >100 μM) were assigned an IC_{50} of 500 μM ($\log(1/IC_{50}) = 3.3$) so they could be included in the analysis. Physicochemical parameters used in the correlations included π, π^2, σ_p, F, R, and MR as published by Hansch.[61] Electronic parameters corresponding to a *p*-phenyl substituent were used, assuming transmission of effects across the ring to the quinoxaline NH. MR was multiplied by 0.1 to place it on a scale similar to that of the other parameters. An indicator variable to denote a PNQX versus iPNQX analog was included in the initial correlations. Since this variable was not found to be significant in any of the correlations, its use was discontinued and it is not reported here. Pairwise correlations between the remaining parameters are shown in Table 2.

Multiple regression analysis using the set of six parameters on the 15-compound set produced eq 1.

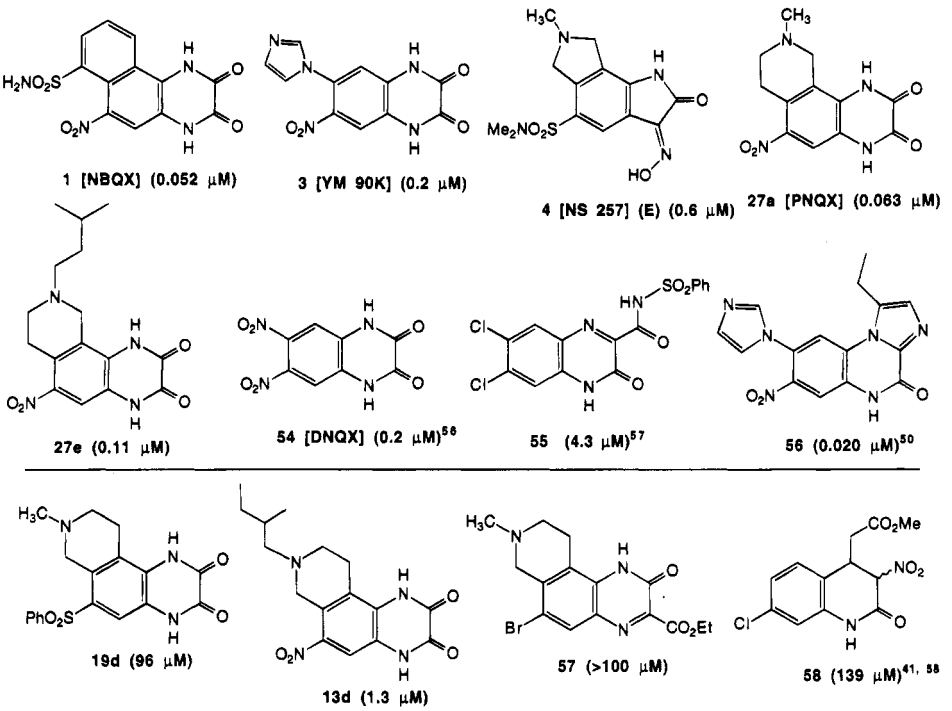
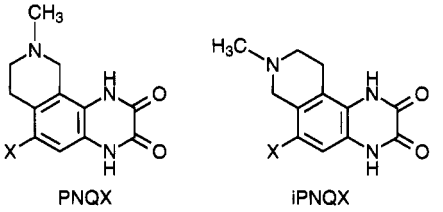


Figure 2. Potent (top) and marginally active (bottom) AMPA antagonists used to define the pharmacophore model.

Table 1. Data Used To Formulate the QSAR



PDNUM	compd	X	series	π	σ	F	R	MR	IC_{50} (μM)	log(1/IC_{50})	calcd (eq 1)	residual
148115	**12b**	H	iPNQX	0.00	0.00	0.00	0.00	0.10	6.5	5.10	4.71	0.39
151002	**25a**	H	PNQX	0.00	0.00	0.00	0.00	0.10	18	4.74	4.71	0.03
147542	**11a**	Br	iPNQX	0.86	0.23	0.73	−0.18	0.89	2.4	5.62	6.10	−0.48
151153	**26b**	Br	PNQX	0.86	0.23	0.73	−0.18	0.89	0.57	6.24	6.10	0.14
154638	**19a**	CH_3	iPNQX	0.56	−0.17	−0.07	−0.11	0.57	18	4.75	4.49	0.26
156492	**30**	CN	PNQX	−0.57	0.66	0.85	0.18	0.63	1.4	5.85	5.86	−0.01
153924	**19b**	CO_2CH_3	iPNQX	−0.01	0.45	0.45	0.19	1.29	44	4.36	4.80	−0.44
154769	**19c**	CO_2H	iPNQX	−0.32	0.45	0.34	0.11	0.69	56	4.25	4.85	−0.60
154405	**29**	$NHCOCH_3$	PNQX	−0.97	0.00	0.47	−0.27	1.49	500	3.30	4.23	−0.93
154404	**28**	NH_2	PNQX	−1.23	−0.66	0.04	−0.68	0.54	100	4.00	3.89	0.11
152371	**13a**	NO_2	iPNQX	−0.28	0.78	1.11	0.16	0.74	0.30	6.52	6.48	0.04
152247	**27a**	NO_2	PNQX	−0.28	0.78	1.11	0.16	0.74	0.080	7.10	6.48	0.62
154770		$PO(OC_2H_5)_2$	iPNQX	−0.10	0.53	0.37	0.19	3.12	500	3.30	3.24	0.06
154767		PO_3H_2	iPNQX	−1.59	0.42	0.34	0.08	1.26	51	4.29	3.83	0.46
154445	**19d**	$SO_2C_6H_5$	iPNQX	0.27	0.70	0.56	0.18	3.32	96	4.02	3.67	0.35

Calculated potencies and residuals from eq 1 are shown

$$\log(1/IC_{50}) = 2.09(\pm 0.35)F - 0.70(\pm 0.14)MR + 0.38(\pm 0.19)\pi + 4.69 \quad (1)$$

$$n = 15, \quad r^2 = 0.84, \quad F = 19.2, \quad s = 0.25$$

in Table 1; a plot of measured versus calculated potency is shown in Figure 5. The QSAR analysis revealed that AMPA potency is strongly influenced by the "field effect" electronic parameter F, such that increased affinity is associated with increasing electron withdrawal. Replacement of F in eq 1 by σ resulted in a significantly inferior correlation ($r^2 = 0.71$). This suggests that the electronic effect is predominately a local effect on the receptor rather than electron withdrawal that is transmitted through the ring system. This idea is supported by the modeling study (*vide infra*), in which it was determined that the nitro and sulfonamide groups in common to the most potent analogs were consistently twisted out of the plane of the ring system for steric reasons, thereby diminishing the resonance contribution.

Beyond the strong electronic influence, potency is modulated by the size of the 6-substituent (smaller groups are associated with increased potency) and, marginally, lipophilicity (potent analogs contain more lipophilic groups at X). Lipophilicity may in fact be describing transport effects (change of overall log P) of these CNS agents. The combination of requirements

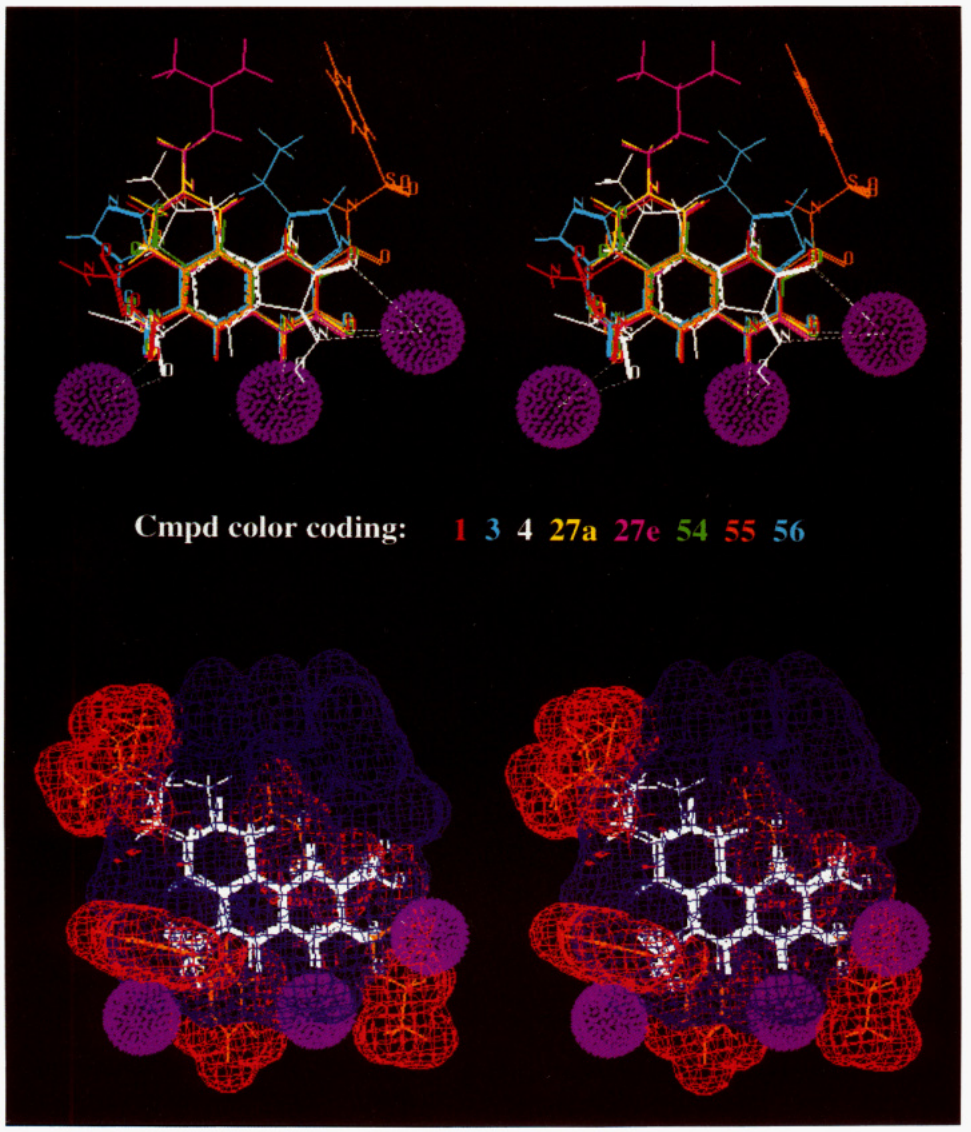


Figure 3. (Top) Overlay of eight active AMPA antagonists used to define the pharmacophore model. Receptor interaction sites appear as purple spheres of dots. White dashed lines denote hydrogen bonding or coulombic interactions with these sites. Compound color coding is given beneath the upper figure. (Bottom) Overlay of four less potent or inactive analogs used in the initial definition of areas of excluded volume. The union of the volumes of the eight active antagonists is in blue; areas of excluded volume are shown in red. The majority of each structure is colored white; that portion that projects outside the active volume has been colored orange.

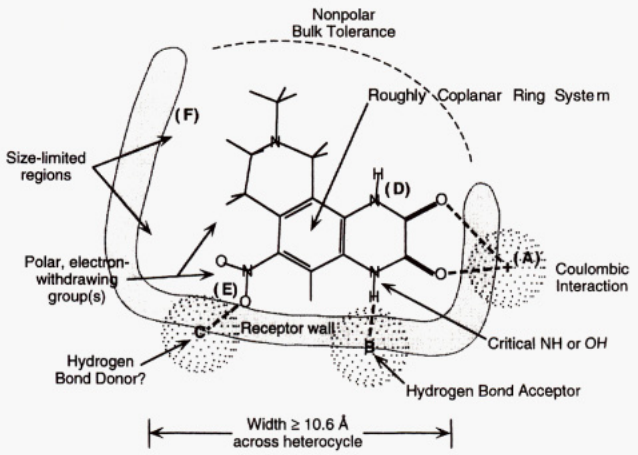


Figure 4. Schematic of the AMPA pharmacophore model.

for electron withdrawal, small size, and increased lipophilicity limits which substituents are tolerated at this position and suggests that large increases in potency with other groups would not be expected beyond the potent nitro derivatives. This has served as a rationale for deemphasizing synthesis of additional substituents at X relative to other regions within the structures.

Table 2. Correlation Matrix (r Values) for Parameters Employed in the Regression Analyses

	log (1/IC_{50})	π	π^2	σ	F	R	MR
log(1/IC_{50})	1	0.29	−0.22	0.40	0.66	0.23	−0.48
π		1	−0.60	0.11	0.10	0.15	0.04
π^2			1	−0.34	−0.14	−0.49	−0.06
σ				1	0.75	0.88	0.40
F					1	0.38	0.14
R						1	0.31
MR							1

Biological Evaluation

Initial evaluation of receptor affinity used [^{3}H]AMPA and [^{3}H]kainate competition binding experiments. Affinity at displacing [^{3}H]AMPA binding was the primary measure used to direct new synthesis and for selection

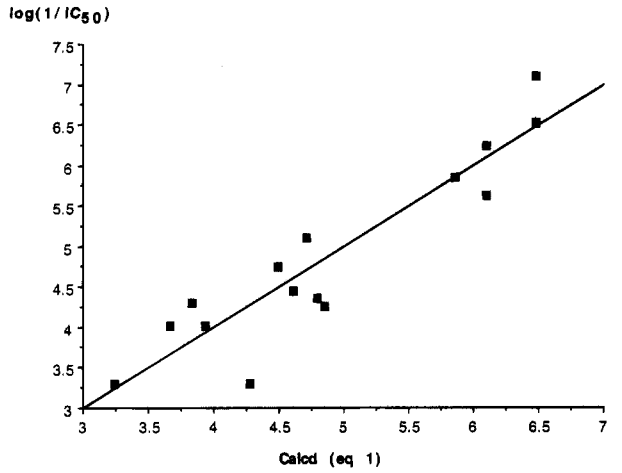


Figure 5. Measured versus calculated (eq 1) potency at AMPA receptors.

of more detailed pharmacological evaluation. As mentioned, [^{3}H]AMPA binding is a strong indicator that a compound will have neuroprotective activity, if it can penetrate into the CNS. The utility of [^{3}H]kainate as an indicator of therapeutic utility remains undefined. The [^{3}H]AMPA assay detects both high- and low-affinity AMPA populations, while the [^{3}H]kainate assay is specific for the low-affinity kainate site. Washed membranes from a rat forebrain synaptosomal preparation were used in both assays. Competition with 10 nM [^{3}H]-AMPA was carried out in a 50 mM Tris buffer containing the chaotropic ion potassium thiocyanate (10 mM) which has been shown to increase AMPA-subtype binding sensitivity.[62] In the kainate binding assay, displacement of 5 nM [^{3}H]kainate from a low-affinity binding site was characterized in 50 mM Tris buffer containing 20 mM calcium.[63,64] Compounds with high affinity for AMPA were also tested in NMDA specific [^{3}H]glycine binding.[65] Results are shown in Table 3.

Results and Discussion

In accordance with previous series of AMPA antagonists,[9,45] the nitro moiety at C-6 provided a significant boost in receptor affinity at both the AMPA and kainate sites and increased glycine-site affinity in both the 8-aza and 9-aza series (NO_2 > Br > H). In the 9-aza series, there was an approximate 10-fold difference in potency at AMPA receptors between the 6-nitro and 6-bromo derivatives, and another 20-fold difference between the 6-bromo and the 6-H derivatives. The 200-fold difference in affinity between the 6-nitro and the 6-H derivatives is the same as reported between **1** and its desnitro counterpart.[45] In the 8-aza series, the affinity differences between 6-nitro, 6-bromo, and 6-H derivatives were smaller but still significant for the *N*-methyl derivatives. These data, together with the QSAR analysis on the (i)PNQX series, indicate that an electron-withdrawing function on the aromatic ring strongly enhances receptor affinity. Furthermore, the modeling study suggested that a nitro group at this position provides a specific nonbonding interaction with the receptor.

In general, variation in the size of the alkyl chains in the 9-aza series (Table 3, series II) from *N*-methyl to *N*-isopentyl did not affect binding dramatically. Among the 6-nitro derivatives, the *N*-methyl analog **27a** (PNQX)

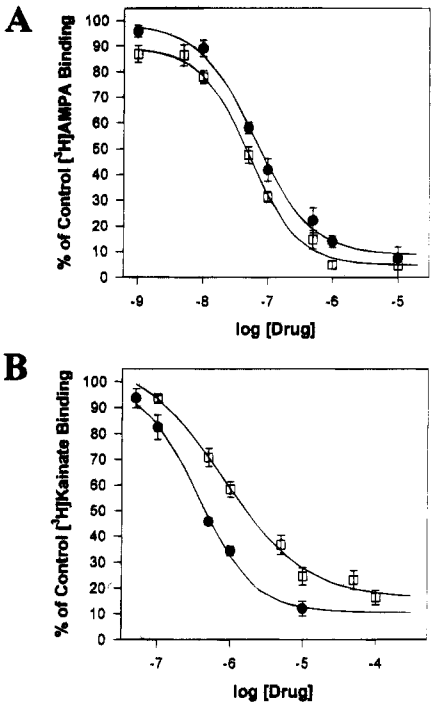


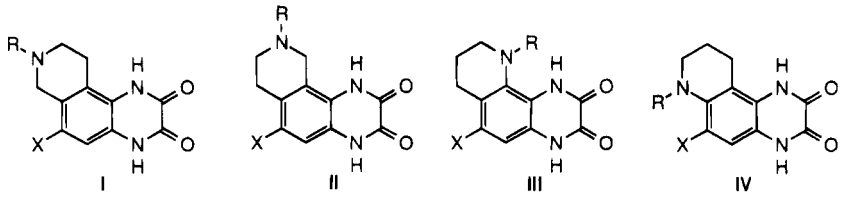
Figure 6. Inhibition of (A) [^{3}H]AMPA and (B) [^{3}H]kainate binding by PNQX (●) and NBQX (□) to rat brain membranes. The calculated IC_{50}s were 51.8 and 63.1 nM for NBQX and PNQX in the [^{3}H]AMPA binding assay and 793 and 368 nM for these compounds in the [^{3}H]kainate binding assay (n = 3−12 experiments per point).

was the most potent AMPA ligand (IC_{50} = 63 nM, Figure 6) found in this study, with affinity comparable to that of **1** (IC_{50} = 52 nM). Replacement of the methyl group with ethyl, isopropyl, *sec*-butyl, or isopentyl decreased affinity within a 3-fold range, indicating that there is little steric interference due to the change in group size at this position. In general, the 8-aza series (Table 3, series I) had lower affinity for AMPA receptors relative to the 9-aza series. Within 8-aza analogs, the size of the *N*-alkyl side chain had a significant influence on affinity (compare **12b−f**, Table 2), with a 20-fold difference in potency noted between the (optimal) *N*-methyl and *N*-heptyl analogs. Thus, there appears to be a steric constraint in the region around N-8, as shown by the modeling study.

For intrinsic AMPA binding affinity, the parent compound **35** in the 10-aza series had greater affinity than the parents **38**, **12a**, or **25a** 7-, 8-, or 9-aza series, respectively. As mentioned in the Chemistry section, attempts at preparing the 6-nitro analog gave a mixture of the 6-nitro and 6-H derivatives. Biological testing of this mixture did not reveal an increase in affinity at the AMPA receptor compared to that of **35** (data not shown).

For most derivatives, the level of AMPA and low-affinity kainate binding were similar, with IC_{50} values within a range of 2- or 3-fold from each other. For the nitro derivatives, an increase in affinity for AMPA relative to kainate receptors was noted. Interestingly, the 6-bromo derivatives **26c**, **26d**, and **26e** containing a larger *N*-alkyl moiety were found to be 6−14-fold kainate selective, suggesting that differences in hydrophobic interactions may exist between the kainate and AMPA receptors. It is also interesting to note that the bromo analogs **26c** and **26d** are more potent at the

Table 3. Inhibition of Receptor Binding in Rat Brain Synaptic Membranes



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compd	series	R	X	IC_{50} (μM)[a] [^{3}H]AMPA	[^{3}H]kainate	[^{3}H]glycine
1 (NBQX)				0.052	0.079	>100
11a	I	Me	Br	1.86 ± 0.58	1.76 ± 0.96	4.3
11b	I	Et	Br	1.3	14	267
11c	I	*n*-Pr	Br	1.1	6.4	>10
11e	I	Me	6-Br,5-NO_2	1.9	5.7	>10
12a	I	H	H	12	17	
12b	I	Me	H	6.5 ± 1.5	29.16 ± 1.72	>100
12c	I	Et	H	21	37	
12d	I	*n*-Pr	H	18	23	
12e	I	$CH_2CH(CH_3)CH_2CH_3$	H	>100	48	>100
12f	I	C_7H_{15}	H	>100	45	>100
13a	I	Me	NO_2	0.29 ± 0.027	0.38 ± 0.069	4.9
13b	I	Et	NO_2	0.54	0.49	5
13c	I	*n*-Pr	NO_2	2.8	1.7	20
13d	I	$CH_2CH(CH_3)CH_2CH_3$	NO_2	1.3	15	>100
13e	I	C_7H_{15}	NO_2	5.8	79	>10
19a	I	Me	Me	17.6	6.7	>10
19b	I	Me	CO_2Me	44	22	
19c	I	Me	CO_2H	56	36	
19d	I	Me	SO_2Ph	96	72	
25a	II	Me	H	40.35 ± 14.65	71.23 ± 9.19	>100
25b	II	Et	H	41	20	
25d	II	$CH(CH_3)CH_2CH_3$	H	>100	21	
25e	II	$(CH_2)_2CH(CH_3)_2$	H	123	25	
25f	II	$CH_2CH(CH_3)CH_2CH_3$	H	>100		
26	II	H	Br	3.5	1.5	1.0
26a	II	Me	Br	2.84 ± 0.51	2.83 ± 0.89	4.0
26b	II	Et	Br	0.74	6.4	0.62
26c	II	$CH(CH_3)CH_2CH_3$	Br	2.5	0.43	>10
26d	II	$(CH_2)_2CH(CH_3)_2$	Br	9.7	0.68	>10
26e	II	$CH_2CH(CH_3)CH_2CH_3$	Br	>100	2.9	>100
27a (PNQX)	II	Me	NO_2	0.063 ± 0.012	0.368 ± 0.005	0.37
27b	II	Et	NO_2	0.166 ± 0.039	0.52 ± 0.046	1.4
27c	II	*i*-Pr	NO_2	0.153 ± 0.011	0.29 ± 0.086	0.93
27d	II	$CH(CH_3)CH_2CH_3$	NO_2	0.28	0.85	0.93
27e	II	$(CH_2)_2CH(CH_3)_2$	NO_2	0.11	1.18	0.86
27f	II	$CH_2CH(CH_3)CH_2CH_3$	NO_2	0.28	0.28	1.3 ± 0.09
28	II	Me	NH_2	~100	28	>100
29	II	Me	NHAc	>100	>100	>100
30[b]	II	Me	CN	1.36	2.4	1.4 ± 0.1
35	III	H	H	2.2	11	1.3
38	IV	H	H	24	8.9	
42	IV	H	Br	9		
46	IV	Me	H	24		
53	IV	$COCO_2Et$	H	13	20	50

[a] The IC_{50} values are mean values for a minimum of two experiments run in duplicate. The accuracy of each assay is reflected by SEM of PNQX: SEM for PNQX in the [^{3}H]AMPA binding assay (n = 4) is ± 0.012 μM; SEM for PNQX in the [^{3}H]kainate binding assay (n = 4) is ± 0.005 μM; SEM for PNQX in the [^{3}H]glycine binding assay (n = 3) is ± 0.12 μM. [b] NMR and MS data were as expected for this compound, while the elemental analysis was poor. The data may not reflect the pure compound.

kainate site than their nitro counterparts **27d** and **27e**, consistent with the importance of hydrophobic interactions to kainate receptor binding.

The N-substituted 8-aza analogs (**11**–**19**) were weak at displacing glycine binding, consistent with a limited steric tolerance in this region that has been postulated in models of the NMDA glycine site[41] (Figure 1). Thus, the most potent compounds in this series (**11a** and **13a**, IC_{50}s = 4.3 and 4.0 μM, respectively) possessed small *N*-alkyl groups. In contrast, a number of compounds in the 9-aza series had affinities < 1 μM at the glycine site (Table 3, series II). Most potent among these was **27a**, with an IC_{50} of 0.37 μM. Similar to the AMPA site, potency was not severely affected by increases in alkyl group size at N-9. Consistent with previous publications on glycine-site antagonists, the nitrile derivative **30** retained moderate potency at both the AMPA and glycine sites.

High-affinity AMPA ligands were profiled in functional assays to determine if they were functioning as antagonists. Using an excitotoxic cell culture assay in rat fetal cortical cultures, compounds were assessed for their ability to block AMPA-induced cell death. Efflux of the enzyme lactate dehydrogenase (LDH)[66,67] has

Table 4. Characterization of AMPA Antagonism in Functional Assays

compd	R	X	IC_{50} (μM) LDH–AMPA[a]	LDH–kainate[b]	cortical wedge[c]
1 (NBQX)			3	2	0.12
13a	Me	NO_2	18	9	1.8
13b	Et	NO_2	25	26	14
26e	$(CH_2)_2CH(CH_3)_2$	Br	>30	>30	>30
26d	$CH(CH_3)CH_2CH_3$	Br	>30	>30	16.5
27a (PNQX)	Me	NO_2	2.5	4	0.5
27b	Et	NO_2	6	7	0.7
27c	*i*-Pr	NO_2	6	7	1.5
27d	$CH(CH_3)CH_2CH_3$	NO_2	15		1.6
27e	$(CH_2)_2CH(CH_3)_2$	NO_2	5	7	
27f	$CH_2CH(CH_3)CH_2CH_3$	NO_2	8	11	

[a] Concentration of compound that inhibits by 50% LDH released by 24 h application of 100 μM AMPA. [b] Concentration of compound that inhibits by 50% LDH released by 24 h application of 500 μM kainate. [c] Concentration of compound that inhibits by 50% depolarizations induced by 10 μM AMPA. The IC_{50} values for LDH–AMPA and LDH–kainate release are mean values for a minimum of two experiments run in triplicate. The accuracy of each assay is reflected by SEM of PNQX: SEM for PNQX in the LDH–AMPA assay ($n = 3$) is $\pm$ 0.19 μM; SEM for PNQX in the LDH–kainate assay ($n = 4$) is $\pm$ 1.9 μM. The cortical wedge IC_{50} values are calculated from a series of three to eight data points, with each data point the average of two to eight experients. The accuracy of each assay is reflected by SEM of data points for PNQX IC_{50} as shown in Figure 8.

been shown to be linearly proportional to neuronal cell damage and death. In this study, compounds with significant affinity at the AMPA site were also found to prevent AMPA-induced LDH release, illustrating that they are neuroprotective *in vitro*. Low-affinity kainate selective compounds **26d** and **26e** were unable to block either AMPA- or kainate-induced LDH release.

Selected compounds were tested for their ability to inhibit AMPA-induced depolarizations in a rat cortical wedge model[68] (Table 4). Reproducible depolarizations were produced using 10 μM AMPA, a concentration that produced depolarizations with a peak amplitude approximately 50% of maximum determined from concentration response curves. AMPA-induced depolarizations were conducted in the presence of 0.5 μM tetrodotoxin (TTX), which prevents the spontaneous release of glutamate in response to action potentials triggered by the opening of sodium channels. Depolarizations (in millivolts) were compared before and after drug treatment. Compounds with high affinity for the AMPA receptor inhibited AMPA-induced depolarizations in a dose-related manner.

While **27a** and **1** were nearly equipotent against AMPA-induced LDH release in cultured cortical neurons (Table 4, Figure 7a), only **27a** was able to block glutamate-induced cell death (Figure 7b), presumably via antagonism of NMDA receptors. **27a** and **1** were also shown to inhibit AMPA-induced depolarizations in the rat cortical wedge (Figure 8a). In this preparation **27a**, but not **1**, was able to inhibit spontaneous epileptiform discharges (SEDs), which are dependent upon NMDA receptor activation (Figure 8b). The inhibition of SEDs by **27a** was reversed by the addition of 1 mM glycine, consistent with the fact that this compound also is a glycine-site antagonist. This suggests that a compound such as **27a**, with a balanced binding profile at the AMPA ($IC_{50} = 0.063$ μM), kainate (0.37 μM), and glycine (0.37 μM) sites, may be superior in functional tests than a compound such as **1** with widely different affinities at these sites.

Blockade of maximal electroshock seizures (MES) was used as a measure of *in vivo* activity. **27a** was significantly more potent than **1** in maximal electroshock (Figure 9a, ED_{50} of 0.44 mg/kg versus 13.1 mg/kg), even though their AMPA affinities are very similar. Once again, activity as an NMDA glycine-site antagonist may

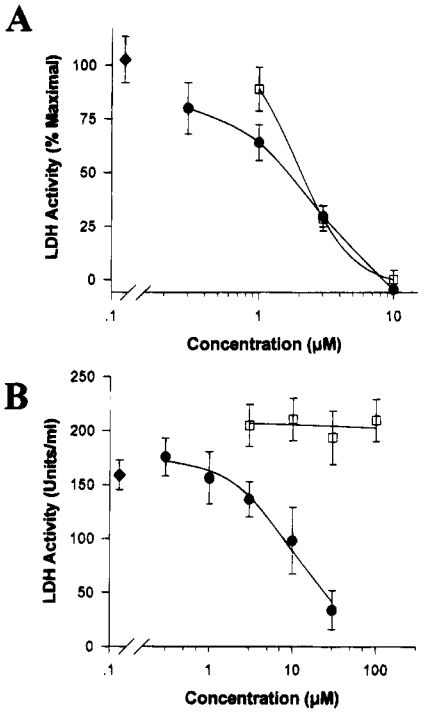

Figure 7. (A) Concentration–response curves for the inhibition of AMPA-induced excitotoxicity in cultured cortical neurons by PNQX (●) and NBQX (□). Data are normalized to sister cultures exposed to 100 μM AMPA (◆) for 24 h. IC_{50}s were 2.6 and 1.9 μM for PNQX and NBQX, respectively (n = 6–15). (B) Concentration–response curves for the inhibition of glutamate-induced excitotoxicity in cultured cortical neurons by PNQX (●) and NBQX (□). The IC_{50} for PNQX was 10 and >100 μM for NBQX (n = 6–9). Control sister cultures were exposed for 5 min to 500 μM glutamate (◆). Under these conditions only NMDA antagonists are effective at reducing cell death.

explain the greater potency of **27a**, since MES is more responsive to NMDA antagonism than AMPA antagonism. This hypothesis is supported in part by comparison of the anticonvulsant activity of **13a** (iPNQX, IC_{50}s = 4.9 μM in [^{3}H]Gly binding and 0.29 μM in [^{3}H]AMPA binding) and **27c** (IC_{50}s = 0.93 μM in [^{3}H]Gly binding and 0.15 μM in [^{3}H]AMPA binding) that parallel their activity at the glycine site. **27c** was active at 6 mg/kg (iv), whereas **13a** was active at 30 mg/kg. Other analogs showed a similar trend, with compounds from the PNQX

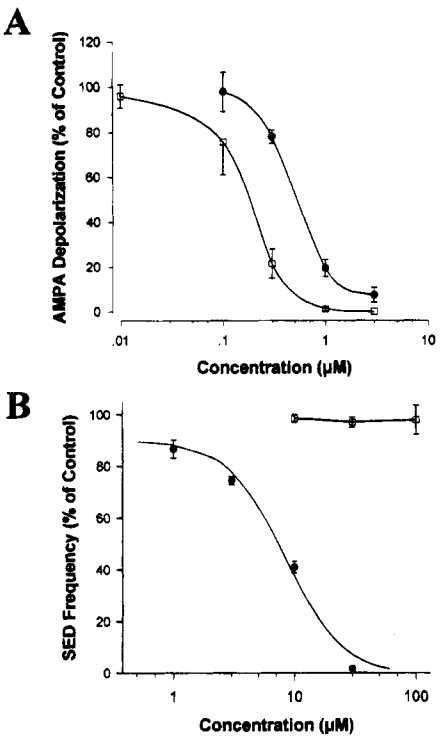

Figure 8. (A) Inhibition of AMPA-induced depolarizations by PNQX (●) and NBQX (□) in rat cortical wedges. IC_{50}s were 0.38 and 0.20 μM for PNQX and NBQX, respectively ($n = 4-8$). (B) Inhibition of spontaneous epileptiform discharges (SEDs) by PNQX (●) and NBQX (□) in rat cortical wedges. SEDs were produced by removal of extracellular Mg^{2+} from the media and have been shown to depend exclusively on NMDA receptor activation. The inhibition of SEDs by PNQX ($IC_{50} = 8.4$ μM) was reversible by addition of 1 mM glycine, indicating an action at the glycine site of the NMDA receptor ($n = 4$).

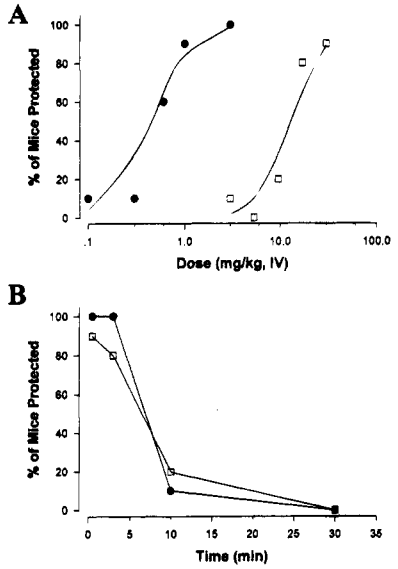

Figure 9. (A) Dose–response curves for PNQX (●) and NBQX (□) in the maximal electroshock model. Compounds were administered intravenously (iv) 30 s before delivery of the shock. (B) Duration of anticonvulsant action for 3 mg/kg PNQX (●) and 25 mg/kg NBQX (□). Doses were chosen based upon >80% inhibition of seizures at peak time of action (30 s) as indicated in A ($n = 10$ animals per dose or time).

series exhibiting higher affinity at both the AMPA and glycine sites, with a corresponding improvement in anticonvulsant activity.

The time course of anticonvulsant activity for **27a** (3 mg/kg, iv) and NBQX (25 mg/kg, iv) was nearly identical. As shown in Figure 9b, at 5 min **27a** protected 100% of mice compared to 80% protection for **1**. At 10 min, both had dropped to less than 25% protected, and at 30 min neither showed any protection. In a model of AMPA-induced neurodegeneration in rat pup, both gave 50% protection at a dosing paradigm of 3 × 20 mg/kg.

Conclusions

A series of quinoxalinediones containing an angular fused piperidine ring have been prepared that have essentially validated the original hypothesis that a chimera of the quinoxalinedione moiety of **1** and the fused azacycloalkyl moiety of **4** would result in potent AMPA antagonists. Subsequent testing of selected analogs has resulted in the idea that a relatively balanced binding profile at AMPA, glycine, and kainate receptors may be optimal in achieving significant *in vivo* potency in animal models of stroke. From this exercise, a compound (**27a**; PNQX) has been identified with moderate to high affinities at the AMPA, glycine, and kainate sites, which has translated into neuroprotective activity in functional assays designed for both AMPA and NMDA receptors. Additionally, this analog was found to have anticonvulsant activity superior in MES than the selective AMPA antagonist **1** (NBQX). The improved *in vivo* activity of **27a** compared to that of **1** indicates that it has potential as a development candidate for acute neuroprotection following stroke.[2]

Two compounds, **26d** and **26e**, were identified that have kainate selectivity relative to their AMPA affinity. These compounds may be useful as biological tools for characterizing kainate receptor activity in normal brain function and disease.

An understanding of the observed SAR, both of compounds reported herein, and AMPA antagonists reported in the literature, has been aided by computational techniques. Thus, a QSAR analysis, run on 6-substituted (i)PNQX analogs, has made possible the quantitative prediction of potency of additional analogs and has led to the conclusion that large increases in potency within the (i)PNQX series over the nitro analog would not be expected with changes in substitution at the 6-position. Development of an AMPA pharmacophore model encompassing a number of different series has facilitated the rationalization of the observed SAR and is being used in the design of additional novel antagonists. Differences in structural requirements between the AMPA and glycine sites within the NMDA receptor complex have been identified. Most important are the more demanding steric limitations of the glycine site in the northwest are as shown in Figure 1. Utilization of the structural features outlined in the receptor models described should facilitate the design of either selective or mixed antagonists with a range of pharmacological properties.

Experimental Section

AMPA, Kainate, and Glycine Receptor Binding. Receptor binding was measured as percent displacement of [^{3}H]-AMPA, kainate, or glycine from extensively washed rat cortical synaptosomal membranes. Assay conditions were adapted from literature methods and were briefly as follows: 10 nM [^{3}H]AMPA, in 50 mM Tris-HCl, pH 7.4, containing 10 mM KSCN, was incubated on ice for 60 min;[62] 5 nM [^{3}H]kainate in 50 mM Tris-HCl, pH 7.4, containing 20 mM $CaCl_2$, was incubated on ice for 90 min;[63,64] 20 nM [^{3}H]glycine, in 50 mM Hepes KOH, pH 7.4, was incubated on ice for 30 min.[65]

Binding assays were terminated by rapid vacuum filtration over Brandell GF/B filters, followed by two 2 mL washes with appropriate buffer.

Lactate Dehydrogenase (LDH) Efflux Assay. Mixed cortical cell cultures, containing both neuronal and glial elements, were prepared as generally described from fetal rats at 18 days gestation.[67] Dissociated cortical cells were plated on 12-well plates (5×10^6 cells/3.8 cm^2) in minimum essential medium (MEM-Earle's salts) supplemented with 10% heat-inactivated horse serum, 10% fetal bovine serum, and glucose (30 mM). Cultures were maintained at 37 °C in a humidified 5% CO_2 atmosphere. After 4 days *in vitro*, non-neuronal cell division was reduced by exposure to 10 μg/mL 5-fluoro-2-deoxyuridine and 25 μg/mL uridine, and the cells were shifted to a maintenance medium similar to the plating media, but lacking fetal serum. Subsequent media replacement was carried out on a biweekly schedule. On day 17 or 18 cells were exposed to 100 μM AMPA or 500 μM kainate for 24 h at 37 °C, or 500 μM glutamate for 5 min at room temperature, replaced with fresh media and returned for incubation at 37 °C for 24 h. For drug testing, compounds are added along with the AMPA, kainate, or glutamate in the exposure media. The following day, neuronal injury was assessed by measurement of LDH released into culture media as previously described.[69] Briefly, 25 μL medium samples were incubated with NADH (reduced α-nicotinamide adenine dinucleotide) for 10 min at 37 °C, after which sodium pyruvate was added to initiate the reaction. Absorbance at 340 nM was measured, from which LDH activity was calculated. The concentration of drug required to inhibit 50% of LDH release resulting from AMPA, kainate, or glutamate treatment alone was calculated using a logit equation.

Preparation and Recording from Cortical Wedge. Wistar rats (90–120 g from Charles River) were killed by decapitation. Brains were maintained in oxygenated Krebs buffer, pH 7.4 (118 mM NaCl, 3.7 mM KCl, 2.6 mM $CaCl_2$, 2.1 mM KH_2PO_4, 25 mM $NaHCO_3$, and 10 mM glucose with 2 mM $MgSO_4$), at approximately 10 °C for dissection and slicing. Coronal slices 400–600 μm thick were sectioned into wedges 1.5–2.0 mm wide at the cortex side and tapering to a point at the white matter side. Each wedge was placed in a two-chambered superfusion bath which allowed separation of the cortex and white matter by a grease seal.[68] Both chambers were superfused (2 mL/min) with Mg^{2+}-free buffer at room temperature. After 90 min of equilibration, differential dc recordings showed spontaneous epileptiform discharges (SEDs) with a frequency (3–4/min), amplitude (0.1–0.6 mv) and afterpotentials that were constant for 5 h or more. In SED experiments, the frequency of SEDs was quantified in three consecutive 10 min control recordings. Drugs effects are reported as percent of control after 30 min treatment.

AMPA-induced depolarizations were obtained with action potentials (and SEDs) blocked by tetrodotoxin (0.5 μM). AMPA was introduced to the cortical chamber at a concentration (10 μM) that produced depolarizations with a peak amplitude approximately 50% of maximum determined from concentration–response curves. Normal buffer was reintroduced after the peak depolarization was observed (usually 4 min). Repeated responses (every 20 min) were obtained with AMPA, and then cortex was continuously superfused with drug. After a 30 min drug treatment, agonist was added in the presence of drug. Responses before and after drug were compared (% of control). Inhibitory effects are reported as the concentration which inhibits 50% of the depolarization (IC_{50}).

Maximal Electroshock Anticonvulsant Assay. Testing for the ability of a compound to inhibit electroshock-induced convulsions in the mouse was adapted from previously published methods.[70] Briefly, mice received an iv dose of compound between 0.1 and 100 mg/kg. At times ranging between 30 s and 60 min following drug administration, a 50 mA, 200 ms long shock was applied via corneal electrodes covered with electrolyte jelly. This suprathreshold shock produces tonic extensor convulsions of all extremities, with inhibition of hind-leg extension taken as evidence of protective action. Following investigation of several dose levels and times, ED_{50}s were estimated.

Molecular Modeling. An initial pharmacophore model of the AMPA site within the receptor complex was generated by superimposing the eight structures presented at the top of Figure 2 using version 6.04 of the SYBYL software.[71] Compounds were selected based on their high AMPA receptor affinity and/or their structural diversity. Each structure was built with side chains in extended conformations and flexible rings in pseudochair orientations and minimized using MAXIMIN (conjugant gradient method; gradient convergence criteria used, delta for convergence set to 0.001). CNDO[72,73] charges were added, and all structures were reminimized (including an electrostatic term) using the same criteria as above. Then, 2.8 Å tensors were added to the quinoxaline ring nitrogen in the 4-position (or the hydroxyl oxygen in the case of **4**) and one NO_2 oxygen (or SO_2 oxygen in the case of **4**) to simulate interactions with putative receptor site atoms. A ring centroid was calculated for the left hand phenyl, pyrrole, or piperidine ring present in the tricyclic antagonists as shown in Figure 2. Three aggregates were defined; the quinoxaline nitrogen and its tensor, the NO_2 group and its tensor, and the ring centroid and corresponding ring carbons. The first two aggregates were added to maintain the 2.8 Å bond length between the putative receptor site atoms and the compounds during the minimizations, and the ring centroid was included to maintain relative coplanarity among the antagonists during the fitting process.

Using MULTIFIT,[74] the structures were constrained to superimpose the endpoints of the two tensors, the two keto oxygens (or keto equivalent nitrogen atoms), and the ring centroids. The initial analysis did not employ a reference structure to which the other molecules were fit. Rather, all molecules were allowed to fit each other simultaneously, resulting in a "consensus conformation" (multifit cluster). To avoid distorting these relatively rigid, cyclic structures during fitting, the intermolecular spring force constants for most atoms employed in fitting were reduced to 5 kcal/mol $Å^2$ from the SYBYL default value (20 kcal/mol $Å^2$). A spring force constant of 10 kcal/mol $Å^2$ was used for the receptor site atom on the tensor attached to the quinoxaline ring nitrogen to reflect the relative importance of this interaction for AMPA affinity. Charges were not employed in the MULTIFIT analyses. Each constrained conformation resulting from the MULTIFIT analysis was then allowed to relax by rerunning MAXIMIN without any fitting constraints. By comparing conformations and energies with and without the fitting constraints, an assessment could be made of the degree of conformity of the pharmacophoric groups within each antagonist to the consensus conformation that emerged from the multifit analysis.

Initial MULTIFIT runs employed only tricyclic antagonists containing a ring centroid as described above. Later runs included bicyclic as well a tricyclic analogs, moving the ring centroid to the central aromatic ring within the tricyclic compounds to allow simultaneous fitting of all active antagonists shown in Figure 2. Finally, the MULTIFIT analyses were repeated in which the ring centroid and its aggregate were removed entirely, leaving four fitting constraints. Comparison of the results from the three runs showed little difference in the conformations and orientations of the antagonists. Therefore, the ring centroid and its aggregate were omitted from further runs.

The resulting relaxed structures after the MULTIFIT analyses are shown in Figure 3. Coordinates for two of the fit analogs (**1** and **27a**) are included as supporting information. Given the low fitting constraints used and the relative rigidity of these cyclic structures, little distortion was observed. All final structures have energies within ±3 kcal/mol from the initially constructed, minimized versions. After the initial pharmacophore was defined, less potent analogs shown at the bottom of Figure 2 were added to define volume constraints. For excluded volume calculations, the 6-SO_2Ph (**19d**) and *N*-isopentyl derivatives (**27e**) were modeled in two low-energy conformations, to give an approximate view of overall areas of excluded volume. A more refined definition of volume

tolerances in the model will emerge as additional compounds containing conformationally restricted side chains are prepared.

Data Processing. Correlations, regressions, and plots were run on an IBM 3090 machine using the SAS program package.[75] In eq 1, the figures in parentheses are the standard errors of the regression coefficients. For this equation, n is the number of compounds, r is the correlation coefficient, F is a significance test, and s is the standard error.

Chemistry. General. The NMR spectra were recorded at 93.94 kG (400 MHz for ^1H, 100 MHz for ^{13}C), 70.45 kG (300 MHz for ^1H, 75 MHz for ^{13}C), and 46.97 kG (200 MHz for ^1H, 50 MHz for ^{13}C) in $CDCl_3$, unless otherwise stated. Residual $CHCl_3$ (δ7.24 ppm) and the center line of the $CDCl_3$ triplet (δ77.0 ppm) were used as internal references for ^1H and ^{13}C, respectively. All OH and NH proton assignments were confirmed by D_2O exchange. D_2O was the solvent of choice for target amino acid derivatives, and the chemical shift values are reported relative to sodium 3-(trimethylsilyl)propionate-*2,2,3,3-d*$_4$ (TSP) as internal standard. IR spectra were recorded on a Nicolet MX-1 FT spectrometer. IR and NMR spectra are not generally reported, but all spectra are consistent with the proposed structures. The mass spectra were obtained on a Finnigan 4500 mass spectrometer or a VG Analytical 7070E/HF mass spectrometer. Where analyses are indicated by the symbols of the elements, the results are within 0.4% of the theoretical values; values outside the limits are indicated. All flash chromatography was run using standard flash silica gel 60 (240–400 mesh) as adsorbent. Melting points were observed on Mel-Temp II lab devices and were uncorrected. All starting materials, unless otherwise stated, were commercially available and were used without purification. Some intermediate products were used directly without further purification or characterization. Anhydrous tetrahydrofuran (THF) and anhydrous dimethylformamide (DMF) were purchased from Aldrich and used directly. Diisopropylamine was distilled from calcium hydride.

General Procedure A. *N*-(1,2,3,4-Tetrahydro-2-methyl-5-isoquinolinyl)acetamide (6a). A solution of 5-nitroisoquinoline (200 g, 1.15 mol) in 1 L of DMF was treated with dimethyl sulfate (160 g, 1.27 mol), and the resulting solution was heated to 90 °C until no starting material remained. After cooling, the reaction mixture was concentrated, and the residue was dissolved in 1.5 L of MeOH and hydrogenated over PtO_2 (1.0 g) at 52 psi for 15.5 h. After a standard separation procedure with $CHCl_3$ and aqueous 1 N NaOH, the organic phase was dried over Na_2SO_4, filtered, and evaporated. The residue was purified by silica gel chromatography (10:2 EtOAc:EtOH) and recrystallization from EtOAc to give a white solid (60.6 g, 26% yield), mp 138–139 °C.

***N*-(2-Ethyl-1,2,3,4-tetrahydro-5-isoquinolinyl)acetamide (6b).** A solution of 5-nitroisoquinoline (25 g, 144 mmol) in EtOH (250 mL) was treated with iodoethane (27 g, 172 mmol) and heated at reflux for 24 h. The precipitate which formed upon cooling was collected and after drying was reduced to the product by procedures described in general procedure A to give a white solid (4.42 g, 17%): mp 131 °C. Anal. ($C_{13}H_{18}N_2O$) C, H, N.

***N*-(1,2,3,4-Tetrahydro-2-propyl-5-isoquinolinyl)acetamide (6c).** A solution of 5-nitroisoquinoline (25 g, 144 mmol) and iodopropane (36.7 g, 216 mmol) was treated in a manner similar as described in general procedure A to give the product (6.72 g, 37% yield): mp 123 °C. Anal. ($C_{14}H_{20}N_2O$) C, H, N.

***N*-(2-Acetyl-1,2,3,4-tetrahydro-5-isoquinolinyl)acetamide (6d).** A solution of 5-nitroisoquinoline (1, 44.8 g, 0.257 mol) in AcOH (600 mL) and acetic anhydride (50 mL) was hydrogenated at 62 psi over platinum oxide (0.5 g). The catalyst was removed by filtration and the filtrate concentrated. The residue was purified by silica gel chromatography (10:1 ethyl acetate:EtOH) to give a white solid (11.25 g, 19%). Recrystallization was from THF:isopropyl ether: mp 154–156 °C. Anal. ($C_{13}H_{16}N_2O_2$) H, N; C: calcd, 67.22; found, 66.56.

General Procedure B. *N*-(8-Bromo-1,2,3,4-tetrahydro-2-methyl-5-isoquinolinyl)acetamide (7a). A solution of **6a** (22.6 g, 111 mmol) in trifluoroacetic acid (500 mL) was treated dropwise with a 1 M solution of bromine in AcOH (122 mL). After stirring overnight, the mixture was concentrated and the resulting oil dissolved in EtOAc and washed with aqueous $NaHCO_3$. The organic layer was dried over Na_2SO_4, filtered, and evaporated. The residue was recrystallized from hot EtOAc to give a white solid: mp 189–190 °C. Anal. ($C_{12}H_{15}BrN_2O$) C, H, N.

***N*-(8-Bromo-2-ethyl-1,2,3,4-tetrahydro-5-isoquinolinyl)acetamide (7b). 6b** (3.65 g, 16.7 mmol) was brominated according to general procedure B to give a white solid (2.64 g, 53%): mp 169 °C. Anal. ($C_{13}H_{17}BrN_2O$) C, N; H: calcd, 5.77; found, 5.23.

***N*-(8-Bromo-1,2,3,4-tetrahydro-2-propyl-5-isoquinolinyl)-acetamide (7c). 6c** (6.33 g, 27.2 mmol) was brominated according to general procedure B to give a white solid (6.17 g, 73%): mp 145–147 °C. Anal. ($C_{14}H_{19}BrN_2O$) C, H, N.

***N*-(2-Acetyl-8-bromo-1,2,3,4-tetrahydro-5-isoquinolinyl)acetamide (7d).** A solution of **6d** (6.67 g, 28.7 mmol) was brominated according to general procedure B. The organic residue was purified on silica gel (4.5:4.5:1 EtOAc:EtOH:NH_4OH) to give a pale pink solid (3.0 g, 34%). An analytical sample was prepared by crystallization from THF/isopropyl ether: mp 192–193 °C. Anal. ($C_{13}H_{15}BrN_2O_2$) C, H, N.

General Procedure C. *N*-(8-Bromo-1,2,3,4-tetrahydro-2-methyl-6-nitro-5-isoquinolinyl)acetamide (8a). A solution of **7a** (19.8 g, 70 mmol) in trifluoroacetic acid (400 mL) was treated dropwise with fuming nitric acid (87 mL). After stirring at room temperature for 4 h, the reaction mixture was concentrated and the residue was treated with a 3:1 EtOAc:THF solution and saturated aqueous $NaHCO_3$. The organic phase was separated, dried over sodium sulfate, filtered, and evaporated. The residue was suspended in THF:isopropyl ether solution and collected by filtration. Recrystallization from hot THF:isopropyl ether gave a beige solid (18.4 g, 80%): mp 190–191 °C. Anal. ($C_{12}H_{14}BrN_3O_3$) C, H, N.

***N*-(8-Bromo-2-ethyl-1,2,3,4-tetrahydro-6-nitro-5-isoquinolinyl)acetamide (8b). 7b** (3.5 g, 11.8 mmol)was nitrated according to general procedure C to give a white solid (2.88 g, 71%): mp 164 °C. Anal. ($C_{13}H_{16}BrN_3O_3$) C, H, N.

***N*-(8-Bromo-1,2,3,4-tetrahydro-2-propyl-6-nitro-5-isoquinolinyl)acetamide (8c). 7c** (5.5 g, 17.7 mmol) was nitrated according to general procedure C to give a white solid (4.41 g, 70%): mp 167–168 °C. Anal. ($C_{14}H_{18}BrN_3O_3$) C, H, N.

***N*-(2-Acetyl-8-bromo-1,2,3,4-tetrahydro-6-nitro-5-isoquinolinyl)acetamide (8d). 7d** (1.0 g, 3.21 mmol) was nitrated using general procedure C to give a tan solid (0.77 g, 68%). Recrystallization was from EtOAc: mp 171 °C. Anal. ($C_{13}H_{14}BrN_3O_4$) C, H, N.

***N*-(8-Bromo-1,2,3,4-tetrahydro-2-heptyl-6-nitro-5-isoquinolinyl)acetamide (8f). 8d** (10.06 g, 28 mmol) was refluxed in 6 N HCl for 2.5 h. The suspension was cooled in ice, filtered, washed with ether, and air-dried to give a yellow solid (8.4 g, 96%). The product (3.0 g, 9.7 mmol) was suspended in 90 mL of 2:1 MeOH:H_2O and treated with *n*-heptyl aldehyde (2.16 g, 18.9 mmol) and $NaCNBH_3$ (1.80 g, 28.6 mmol). After stirring for 1 h, an additional 1.0 g each of the aldehyde and $NaCNBH_3$ was added. The reaction mixture was stirred for an additional 2 h at 25 °C, then concentrated, and quenched with 10% $NaHCO_3$ solution, and the product was extracted with $CHCl_3$. The combined organic extracts were dried and concentrated, and the residue was chromatographed on silica gel (gradient elution with $CHCl_3$ followed by 20% EtOAc:$CHCl_3$). The product was triturated with heptane:ether, filtered, and dried to give **8f** as a yellow solid (3.1 g, 79%).

***N*-(8-Bromo-1,2,3,4-tetrahydro-2-(1-(2-methylbutyl))-6-nitro-5-isoquinolinyl)acetamide (8e).** The hydrolysis product from **8d** (3.0 g, 9.72 mmol) was treated with 2-methylbutyl aldehyde (1.67 g, 19.4 mmol) and $NaCNBH_3$ (1.83 g, 29.2 mmol) in a manner similar to that described for the preparation of **8f** to give product (3.2 g, 87%).

General Procedure D. 8-Bromo-1,2,3,4-tetrahydro-2-methyl-6-nitro-5-isoquinolinamine. A solution of **8a** (3.68 g, 11.2 mmol) in 3:2 sulfuric acid:water (80 mL) was heated at 90 °C for 1 h. After cooling, the mixture was poured onto ice and basified with aqueous NH_4OH. The precipitate was

collected by filtration and dried *in vacuo* to give a yellow solid (3.17 g, 99%).

General Procedure E. 8-Bromo-1,2,3,4-tetrahydro-2-methyl-5,6-isoquinolinediamine (9a). A solution of 8-bromo-1,2,3,4-tetrahydro-2-methyl-6-nitro-5-isoquinolinamine (0.78 g, 2.73 mmol) in THF (100 mL) was treated with Raney nickel (1 g) and hydrogenated for 1.5 h at room temperature. The catalyst was removed by filtration and the filtrate concentrated to give a tan solid (0.63 g, 90%).

8-Bromo-2-ethyl-1,2,3,4-tetrahydro-6-nitro-5-isoquinolinamine. 8b (2.5 g, 7.3 mmol) was hydrolyzed according to general procedure D to give a yellow solid (2.05 g, 82%).

8-Bromo-2-ethyl-1,2,3,4-tetrahydro-5,6-isoquinolinediamine (9b). 8-Bromo-2-ethyl-1,2,3,4-tetrahydro-6-nitro-5-isoquinolinamine (1 g, 3.33 mmol) was hydrogenated according to general procedure E to give an oil (0.88 g, 98%).

8-Bromo-1,2,3,4-tetrahydro-2-propyl-6-nitro-5-isoquinolinamine. 8c (4.24 g, 11.9 mmol) was hydrolyzed according to general procedure D to give a yellow solid (3.68 g, 98%).

8-Bromo-1,2,3,4-tetrahydro-2-propyl-5,6-isoquinolinediamine (9c). 8-Bromo-1,2,3,4-tetrahydro-2-propyl-6-nitro-5-isoquinolinamine (0.98 g, 3.12 mmol) was hydrogenated according to general procedure E to give an oil (0.77 g, 87%).

General Procedure F. 1,2,3,4-Tetrahydro-2-methyl-5,6-isoquinolinediamine (10a). A solution of 8-bromo-1,2,3,4-tetrahydro-2-methyl-6-nitro-5-isoquinolinamine (1.5 g, 5.24 mmol) and 20% Pd on carbon (0.3 g) in MeOH (100 mL) was hydrogenated at 50 psi for 24 h. After removing the catalyst by filtration, the filtrate was concentrated and the residue partitioned between $CHCl_3$ and aqueous $NaHCO_3$. The organic layer was dried over Na_2SO_4, filtered, and evaporated. The residue was purified on silica gel (25% EtOH in EtOAc) to give an oil (0.45 g, 48%).

2-Ethyl-1,2,3,4-tetrahydro-5,6-isoquinolinediamine (10b). 8-Bromo-2-ethyl-1,2,3,4-tetrahydro-6-nitro-5-isoquinolinamine (1 g, 3.33 mmol) was hydrogenated according to general procedure F to give an oil (0.7 g).

1,2,3,4-Tetrahydro-2-propyl-5,6-isoquinolinediamine (10c). 8-Bromo-1,2,3,4-tetrahydro-2-propyl-6-nitro-5-isoquinolinamine (1.5 g, 4.77 mmol) was hydrogenated according to general procedure F. The residue was suspended in isopropyl ether and the yellow precipitate (1.27 g, 93%) collected by filtration.

1,2,3,4-Tetrahydro-2-heptyl-5,6-isoquinolinediamine (10e). *N*-(8-Bromo-1,2,3,4-tetrahydro-2-heptyl-6-nitro-5-isoquinolinyl)acetamide (3.1 g, 7.7 mmol) was hydrolyzed according to general procedure D and dissolved in MeOH (500 mL) with 20% palladium on carbon (0.4 g). The reaction mixture was stirred vigorously for 6.5 h at 25 °C under 1 atm of hydrogen. The catalyst was removed by filtration, the solution concentrated, and the residue used quickly in the next reaction.

1,2,3,4-Tetrahydro-2-(1-(2-methylbutyl))-5,6-isoquinolinediamine (10d). The reduction was accomplished using the same methodology as for **10e**. The crude product was used directly in the next step.

General Procedure G. 6-Bromo-1,4,7,8,9,10-hexahydro-8-methylpyrido[4,3-*f*]quinoxaline-2,3-dione (11a). A solution of **9a** (0.62 g, 2.56 mmol) and oxalic acid (0.23 g, 2.56 mmol) in 3 N HCl (25 mL) was heated at reflux for 18 h. After cooling to room temperature, the precipitate was collected by filtration and washed with cold water. The tan solid was recrystallized from hot water and dried *in vacuo* (0.19 g, 20%): mp 315–320 °C dec. Anal. ($C_{12}H_{12}BrN_3O_2$·HCl·H_2O) C, H, N.

6-Bromo-1,4,7,8,9,10-hexahydro-8-ethylpyrido[4,3-*f*]quinoxaline-2,3-dione (11b). 9b (0.88 g, 3.26 mmol) was condensed with oxalic acid as in general procedure G to give a white solid (0.71 g, 59%): mp 325–350 °C dec. Anal. ($C_{13}H_{14}BrN_3O_2$·HCl·0.6H_2O) C, H, N.

6-Bromo-1,4,7,8,9,10-hexahydro-8-propylpyrido[4,3-*f*]quinoxaline-2,3-dione (11c). 9c (0.77 g, 2.71 mmol) was condensed with oxalic acid as in general procedure G to give a white solid: mp 300–320 °C. Anal. ($C_{14}H_{16}BrN_3O_2$·HCl·H_2O) C, H, N.

1,4,7,8,9,10-Hexahydropyrido[4,3-*f*]quinoxaline-2,3-dione (12a). 1,2,3,4-Tetrahydro-5,6-isoquinolinediamine was condensed with oxalic acid as in general procedure G to give a white solid: mp >300 °C. Anal. ($C_{11}H_{11}N_3O_2$·HCl·0.35H_2O) C, H, N, Cl.

1,4,7,8,9,10-Hexahydro-8-methylpyrido[4,3-*f*]quinoxaline-2,3-dione (12b). 10a (0.45 g, 2.54 mmol) was condensed with oxalic acid as in general procedure G to give a white solid (0.46 g, 68%): mp 310–335 °C dec. Anal. ($C_{12}H_{13}BrN_3O_2$·0.95HCl·0.1H_2O) C, H, N.

1,4,7,8,9,10-Hexahydro-8-ethylpyrido[4,3-*f*]quinoxaline-2,3-dione (12c). 10b (0.7 g, 3.66 mmol) was condensed with oxalic acid as in general procedure G to give a white solid (0.4 g, 38%): mp 325–330 °C. Anal. ($C_{13}H_{15}BrN_3O_2$·HCl·0.3H_2O) C, H, N.

1,4,7,8,9,10-Hexahydro-8-propylpyrido[4,3-*f*]quinoxaline-2,3-dione (12d). 10c (1.24 g, 4.33 mmol) was condensed with oxalic acid as in general procedure G to give a white solid (0.83 g, 65%): mp 340–350 °C. Anal. ($C_{14}H_{17}BrN_3O_2$·HCl·0.1H_2O) C, H, N.

1,4,7,8,9,10-Hexahydro-8-(1-(2-methylbutyl))pyrido[4,3-*f*]quinoxaline-2,3-dione (12e). 10d (8.77 mmol) was condensed with oxalic acid in 3 N HCl to give a pale solid (2.1 g, 75%): mp >300 °C. Anal. ($C_{16}H_{21}N_3O_2$·1.86HCl·H_2O) C, N; H: calcd, 6.71; found, 6.21.

1,4,7,8,9,10-Hexahydro-8-heptylpyrido[4,3-*f*]quinoxaline-2,3-dione (12f). 10e (7.44 mmol) was dissolved in 3 N HCl (30 mL) and treated with oxalic acid (1.88 g, 1.5 mmol). After refluxing for 18 h, the reaction mixture was cooled and the precipitate collected by filtration, washed with ether, and air-dried to give a white solid (1.65 g, 65%): mp 308 °C. Anal. ($C_{18}H_{27}N_3O_2$·HCl·0.2H_2O) C, N; H: calcd, 7.94; found, 7.17.

1,4,7,8,9,10-Hexahydro-8-methyl-6-nitropyrido[4,3-*f*]-quinoxaline-2,3-dione Methanesulfonate (13a). A solution of **12a** (1.07 g, 4.7 mmol) was dissolved in sulfuric acid (20 mL). Potassium nitrate (0.51 g, 5.0 mmol) was added in one portion. After stirring for 1 h, the reaction mixture was poured onto ice and basified with NH_4OH. The resulting yellow solid was collected by filtration and washed with water and ether. The solid was suspended in water:DMF and treated with methanesulfonic acid (0.5 g). Solvent was removed and the residue suspended in acetone and collected by filtration and dried (0.6 g, 35%): mp 292–294 °C. Anal. ($C_{12}H_{12}N_4O_4$·CH_4O_3S·0.5H_2O) C, H, N, S.

1,4,7,8,9,10-Hexahydro-8-ethyl-6-nitropyrido[4,3-*f*]quinoxaline-2,3-dione Hydrochloride (13b). The product was obtained from **12b** by an identical method as for **13a** and converted into the hydrochloride salt (0.35 g, 25%): mp 315 °C. Anal. ($C_{13}H_{14}N_4O_4$·1.5HCl·0.5H_2O) C, H, N.

1,4,7,8,9,10-Hexahydro-8-propyl-6-nitropyrido[4,3-*f*]-quinoxaline-2,3-dione (13c). 12c was nitrated as described above to give the product (0.64 g, 60%): mp 301 °C. Anal. ($C_{14}H_{16}N_4O_4$·HCl·0.3H_2O) C, H, N.

1,4,7,8,9,10-Hexahydro-8-heptyl-6-nitropyrido[4,3-*f*]-quinoxaline-2,3-dione Methanesulfonate (13e). 12f (1.0 g) was treated with fuming nitric acid (0.15 mL) in concentrated sulfuric acid (15 mL), stirred for 2 h at 25 °C, and then poured onto 50 g of ice. The solid was collected by filtration and washed first with $CHCl_3$:methanol and then with hot water (10 mL). The solid was suspended in 25 mL of water and basified with ammonium hydroxide. The solid was collected, suspended in 15 mL of MeOH, and dissolved with heating. Methanesulfonic acid (1 mL) was added, and upon cooling the precipitate was collected and washed with ether: EtOH to give product as the methanesulfonate salt (0.25 g, 19%): mp 183–185 °C. Anal. ($C_{18}H_{24}N_4O_4$·CH_4O_3S·H_2O) C, H, N.

1,4,7,8,9,10-Hexahydro-8-(1-(2-methylbutyl))-6-nitropyrido[4,3-*f*]quinoxaline-2,3-dione Hydrochloride (13d). 12e (1.0 g) was nitrated as described for **13e**. The product was refluxed in MeOH:HCl, cooled, filtered, and washed with MeOH to give the hydrochloride salt as a white solid (0.61 g, 47%): mp 285 °C. Anal. ($C_{16}H_{20}N_4O_4$·HCl·0.33H_2O) C, H, N.

6-Bromo-2,3-dichloro-8-methyl-7,8,9,10-tetrahydropyrido[4,3-*f*]quinoxaline (14). A mixture of **11a** (1.0 g, 3.22 mmol), $SOCl_2$ (25 mL), and DMF (1.0 mL) was heated at reflux

overnight. The solvent was removed and the residue partitioned between $CHCl_3$ and saturated $NaHCO_3$. The organic layer was dried over Na_2SO_4, filtered, and evaporated to give an orange, brown solid (0.83 g, 74%).

6-Bromo-2,3-dimethoxy-8-methyl-7,8,9,10-tetrahydropyrido[4,3-*f*]quinoxaline (15). Sodium (2.85 g, 124 mmol) was dissolved in MeOH (200 mL) and then treated in portions with the imino chloride **14** (4.44 g, 11.6 mmol). After stirring overnight, the solvent was concentrated to 100 mL and added to CH_2Cl_2:H_2O (400 mL). After extraction, the combined organic layers were dried and concentrated to give the bis(methyl ether) (5.68 g): mp 162 °C. Anal. ($C_{14}H_{16}BrN_3O_2$) C, H, N.

2,3-Bis(benzyloxy)-6-bromo-8-methyl-7,8,9,10-tetrahydropyrido[4,3-*f*]quinoxaline (16). A suspension of sodium hydride (60% dispersion in oil, 15 g, 0.375 mol) in THF (500 mL) was treated with benzyl alcohol (50 mL). After hydrogen evolution ceased, the imino chloride **14** (14.97 g, 39 mmol) was added in portions, and the mixture was stirred at room temperature. The solvent was concentrated to 100 mL and added to CH_2Cl_2:H_2O (500 mL). After extraction, the combined organic layers were dried over K_2CO_3 and evaporated. Residual benzyl alcohol was removed *in vacuo*, and the residue was purified by silica gel chromatography (1:1 EtOAc:$CHCl_3$). The resulting yellow solid was recrystallized from hot CH_3CN (charcoal to decolorize) to give a first crop (7.16 g, 37%): mp 147 °C. Anal. ($C_{26}H_{24}BrN_3O_2$) C, H, N.

2,3-Dimethoxy-6,8-dimethyl-7,8,9,10-tetrahydropyrido[4,3-*f*]quinoxaline (17a). A solution of **15** (0.5 g, 1.48 mmol) in THF (20 mL) was cooled to −78 °C and treated with *s*-BuLi (1.7 mL of 1.17 M in cyclohexane) to give a persistant black green solution. Methyl iodide (0.123 mL) was added, and the mixture was stirred at −78 °C for 30 min and then warmed to room temperature. The reaction was quenched with saturated aqueous NH_4Cl (2 mL), and the mixture was extracted with $CHCl_3$, dried over Na_2SO_4, filtered, and evaporated. The residue was triturated in heptane and collected by filtration to give an orange solid (196 mg, 48%).

2,3-Dimethoxy-8-methyl-6-(phenylsulfonyl)-7,8,9,10-tetrahydropyrido[4,3-*f*]quinoxaline (17b). A solution of **15** (0.5 g, 1.48 mmol) in THF (20 mL) was cooled to −78 °C and treated with *s*-BuLi (1.7 mL of 1.17 M in cyclohexane) to give a persistant black green solution. Benzenesulfonyl fluoride (0.24 mL) was added, and the mixture was stirred at −78 °C for 30 min and then warmed to room temperature. The reaction was quenched with saturated aqueous NH_4Cl, and the mixture was extracted with $CHCl_3$, dried over Na_2SO_4, filtered, and evaporated. The residue was recrystallized from CH_3CN to give a tan solid (109 mg, 29%).

Methyl 2,3-Bis(benzyloxy)-8-methyl-7,8,9,10-tetrahydropyrido[4,3-*f*]quinoxaline-6-carboxylate (18). A solution of **16** (2.4 g, 5.19 mmol), bis(triphenylphosphine)palladium(II) chloride (0.5 g), Et_3N (1.5 g), and MeOH (5 g) in CH_3CN (75 mL) was placed in a high-pressure vessel, charged with carbon monoxide (820 psi), and heated at 100 °C for 64 h. After cooling, the solvent was removed, and the residue was dissolved in $CHCl_3$ and washed with aqueous $NaHCO_3$. Combined organic layers were dried over Na_2SO_4, filtered, and evaporated. The residue was suspended in CH_3CN and collected by filtration to give after drying *in vacuo* a yellow solid (1.17 g, 48%): mp 167−168 °C. Anal. ($C_{28}H_{27}N_3O_4$) C, H, N.

1,4,7,8,9,10-Hexahydro-6,8-dimethylpyrido[4,3-*f*]-quinoxaline-2,3-dione (19a). A suspension of **17a** (0.172 g, 0.63 mmol) in 3 N HCl (4 mL) was heated at reflux for 1 h. A precipitate formed, so an additional 3 N HCl (3 mL) was added and heated to boiling. The solution was treated with activated charcoal and filtered. After cooling to 5 °C, the precipitate that formed was collected by filtration and dried *in vacuo* (83 mg, 43%): mp 323−330 °C. Anal. ($C_{13}H_{15}N_3O_2$·HCl·1.25H_2O) C, H, N, Cl.

Methyl 1,4,7,8,9,10-Hexahydro-8-methyl-2,3-dioxopyrido[4,3-*f*]-6-quinoxalinecarboxylate (19b). A solution of **18** (100 mg, 0.21 mmol) in acetic acid (75 mL) was treated with 20% Pd/C (0.1 g) and shaken on a Parr apparatus under H_2 (50 psi). After removal of the catalyst, the filtrate was evaporated and the residue triturated with diethyl ether and collected by filtration to give a tan solid (42 mg, 69%): mp 290−300 °C. Anal. ($C_{14}H_{15}N_3O_4$·0.5$C_2H_4O_2$·1.3H_2O) C, H, N.

1,4,7,8,9,10-Hexahydro-8-methyl-2,3-dioxopyrido[4,3-*f*]-6-quinoxalinecarboxylic Acid (19c). A suspension of **19b** (0.072 g) was stirred in 1 N NaOH (1 mL). After stirring overnight, the reaction mixture was neutralized with 0.5 mL of 3 N HCl, and the precipitate was collected by filtration, washed with ice water, and dried *in vacuo* to give a white solid (0.051 g): mp >325 °C. Anal. ($C_{13}H_{13}N_3O_4$·HCl) C, H, N.

8-Methyl-6-(phenylsulfonyl)-1,4,7,8,9,10-hexahydropyrido[4,3-*f*]quinoxaline-2,3-dione (19d). A suspension of **17b** (0.158 g, 0.43 mmol) in 3 N HCl (4 mL) was heated at reflux for 1 h. The solvent was removed, and the residue was dissolved in HPLC grade H_2O (4 mL) and then lyophilized to give a white solid (87 mg, 46%): mp 263−270 °C. Anal. ($C_{18}H_{17}N_3O_4S$·HCl·2H_2O) C, H, N.

5-Bromo-8-nitroisoquinoline (21). 5-Bromoisoquinoline (101 g, 0.485 mol) was nitrated according to literature procedure.[46] The crude product was purified on silica gel (chloroform) and triturated in 5% EtOAc in hexane to give a yellow solid (116.7 g, 95%): mp 140−141 °C. Anal. ($C_9H_5BrN_2O_2$) C, H, N, Br.

5-Bromo-1,2,3,4-tetrahydro-2-methyl-8-nitroisoquinoline (22a). 21 (73.2 g, 0.289 mol) was dissolved in $CHCl_3$ (1 L) and cooled to 0 °C. Methyl trifluoromethanesulfonate (50 g, 0.305 mol) was added dropwise to the light orange solution. A precipitate forms after about 1 min. The reaction mixture was stirred for 18 h after addition of the MeOTf was complete. The solid was filtered off, washed with $CHCl_3$, and air-dried to give a light yellow solid (118.5 g, 98%) which was used without further purification.

General Procedure H. The resulting methyl triflate salt of **21** (58.4 g, 0.140 mol) was dissolved in formic acid (250 mL) and cooled in ice bath under a stream of N_2. $NaCNBH_3$ (17.5 g, 0.265 mol) was added portionwise and the reaction mixture stirred for 30 min. The reaction was quenched with water (50 mL) and the volume reduced *in vacuo*. The residue was diluted in water (250 mL), cooled in an ice bath, and basified with NH_4OH. The solid precipitate was filtered, washed with water, dissolved in $CHCl_3$, dried over $MgSO_4$, and evaporated to give a yellow solid (37.26 g, 98%): mp 85−87 °C. Anal. ($C_{10}H_{11}BrN_2O_2$) C, H, N.

5-Bromo-2-ethyl-1,2,3,4-tetrahydro-8-nitroisoquinoline (22b). A solution of **21** (20.0 g, 79 mmol) in THF (400 mL) was cooled to 0 °C and treated sequentially with $NaBH_4$ (21 g, 0.555 mol) and slow addition of AcOH (125 mL). After warming to room temperature, an additional 2 equiv of $NaBH_4$ was added. The mixture was quenched with water, then basified with NaOH (aqueous), and extracted with EtOAc. The organic residue was chromatographed on silica gel (3:1 hexane:EtOAc) to give product (12.1 g, 54%): mp 49−50 °C. Anal. ($C_{11}H_{13}BrN_2O_2$) C, H, N.

5-Bromo-1,2,3,4-tetrahydro-8-nitroisoquinoline (22c). 21 (10.0 g, 39.6 mmol) was dissolved in $CHCl_3$ (100 mL), $MeSO_3H$ (2.2 mL, 33.9 mmol) was added, and the reaction mixture was refluxed for 48 h. The solvent was removed and the residue reduced according to general procedure H using $NaBH_4$ (3.20 g, 86.90 mmol) and formic acid (125 mL) to give a yellow solid (8.64 g, 85%).

General Procedure J. 5-Bromo-2-(1-methylethyl)-1,2,3,4-tetrahydro-8-nitroisoquinoline (22d). 22c (4.13 g, 16.1 mmol) was dissolved in MeOH (300 mL), and then 5 N HCl·MeOH (1 mL) was added, followed by acetone (2.5 mL, 34.05 mmol). $NaCNBH_3$ (1.06 g, 16.02 mmol) was added portionwise, and the reaction stirred at room temperature for 72 h. Additional 5 N HCl·MeOH (1 mL), acetone (1.25 mL), and $NaCNBH_3$ (0.50 g) were added. 5 N HCl·MeOH (1 mL) was again added after 48 and 96 h, after which the reaction was quenched with 10% HCl (50 mL). The MeOH was evaporated, water was added (150 mL), and the reaction mixture was basified with NH_4OH. The aqueous layer was extracted with $CHCl_3$, and the combined organics were washed with brine, dried over $MgSO_4$, filtered, and evaporated. Flash chromatography on silica gel (25% EtOAc:hexanes) gave a yellow solid (4.05 g, 84%).

5-Bromo-2-(1-methylpropyl)-1,2,3,4-tetrahydro-8-nitroisoquinoline (22e). 22c (6.02 g, 23.4 mmol) underwent reductive alkylation using 2-butanone according to general procedure J to give an oil (5.2 g, 71%).

5-Bromo-2-(3-methylbutyl)-1,2,3,4-tetrahydro-8-nitroisoquinoline (22f). 22c (5.36 g, 20.9 mmol) underwent reductive alkylation using isovaleraldehyde according to general procedure J to give an oil (6.93 g, 91%).

General Procedure I. *N*-(5-Bromo-1,2,3,4-tetrahydro-2-methyl-8-isoquinolinyl)acetamide (23a). A solution of **22a** (6 g, 22.1 mmol) in THF (100 mL) was stirred with acetone-deactivated Raney nickel under a hydrogen atmosphere for 3 h. After removing the catalyst, the organic residue was dissolved in acetic anhydride (100 mL) and stirred for 72 h. The solvent was removed, and the solid residue was washed with diethyl ether and then partitioned between $CHCl_3$ and aqueous $NaHCO_3$. The organic layer was dried over $MgSO_4$, filtered, and evaporated to give the product (5.03 g, 80%): mp 192–194 °C. Anal. ($C_{12}H_{15}BrN_2O$) C, H, N, Br.

***N*-(5-Bromo-2-ethyl-1,2,3,4-tetrahydro-8-isoquinolinyl)acetamide (23b). 22b** (7.66 g, 26.9 mmol) was reduced and acetylated according to general procedure I to give the product (4.67 g, 58%) after recrystallization from EtOAc: mp 185–187 °C. Anal. ($C_{13}H_{17}BrN_2O{\cdot}0.2H_2O$) C, H, N.

***N*-(5-Bromo-2-(1-methylethyl)-1,2,3,4-tetrahydro-8-isoquinolinyl)acetamide (23c). 22d** (4.06 g, 13.6 mmol) was reduced and acetylated according to general procedure H to give a white solid (3.42 g, 81%).

***N*-(5-Bromo-2-(1-methylpropyl)-1,2,3,4-tetrahydro-8-isoquinolinyl)acetamide (23d). 22e** (5.72 g, 18.3 mmol) was reduced and acetylated according to general procedure H to give an oil (5.6 g, 95%). Subsequent treatment with Ac_2O yielded a white solid: mp 128–129 °C. Anal. ($C_{15}H_{21}BrN_2O$) C, H, N.

***N*-(5-Bromo-2-(3-methylbutyl)-1,2,3,4-tetrahydro-8-isoquinolinyl)acetamide (23e). 22f** (6.89 g, 21.1 mmol) was reduced and acetylated according to general procedure H to give a white solid (5.38 g, 75%): mp 158–159 °C. Anal. ($C_{16}H_{23}BrN_2O$) H, N; C: calcd, 56.64; found, 57.10.

5-Bromo-1,2,3,4-tetrahydro-2-methyl-7-nitro-8-isoquinolinamine (24a). 23a (4.93 g, 17.4 mmol) was nitrated using general procedure C to give *N*-(5-bromo-1,2,3,4-tetrahydro-2-methyl-7-nitro-8-isoquinolinyl)acetamide as a light brown solid (5.16 g, 90%). Hydrolysis (3.01 g, 9.17 mmol) using general procedure D gave product (2.48 g, 95%). Anal. ($C_{10}H_{12}BrN_3O_2{\cdot}0.35H_2O$) C, H, N.

5-Bromo-2-ethyl-1,2,3,4-tetrahydro-7-nitro-8-isoquinolinamine (24b). 23b (5.11 g, 17.2 mmol) was nitrated using general procedure C to give *N*-(5-bromo-2-ethyl-1,2,3,4-tetrahydro-7-nitro-8-isoquinolinyl)acetamide as a light brown solid (5.61 g, 95%). Hydrolysis (3.07 g, 8.97 mmol) using general procedure D gave product (2.53 g, 94%).

5-Bromo-2-[1-(1-methylethyl)]-1,2,3,4-tetrahydro-7-nitro-8-isoquinolinamine (24c). 23c (3.32 g, 10.7 mmol) was nitrated using general procedure C to give *N*-(5-bromo-1,2,3,4-tetrahydro-2-(1-methylethyl)-7-nitro-8-isoquinolinyl)acetamide as a light brown solid (3.43 g, 90%). Hydrolysis (0.99 g, 2.78 mmol) using general procedure D gave product (0.79 g, 91%).

5-Bromo-2-[1-(1-methylpropyl)]-1,2,3,4-tetrahydro-7-nitro-8-isoquinolinamine (24d). 23d (5.54 g, 17.0 mmol) was nitrated using general procedure C to give *N*-(5-bromo-1,2,3,4-tetrahydro-2-(1-methylpropyl)-7-nitro-8-isoquinolinyl)-acetamide as a light brown solid (4.40 g, 70%): mp 182–183 °C. Anal. ($C_{15}H_{20}BrN_3O_3$) C, H, N. Hydrolysis (2.56 g, 6.91 mmol) using refluxing 6 N HCl for 3 h gave an off yellow solid as the HCl salt (2.37 g, 94%). Treatment with $NaHCO_3$ gave the free base: mp 122 °C. Anal. ($C_{13}H_{18}BrN_3O_2$) C, H, N.

5-Bromo-2-[1-(3-methylbutyl)]-1,2,3,4-tetrahydro-7-nitro-8-isoquinolinamine (24e). 23e (3.16 g, 9.31 mmol) was nitrated using general procedure C to give *N*-(5-bromo-2-(3-methylbutyl)-1,2,3,4-tetrahydro-7-nitro-8-isoquinolinyl)acetamide as a light brown solid (5.48 g, 92%). Hydrolysis (5.00 g, 13.01 mmol) using general procedure D gave product (2.92 g, 70%): mp 114–115 °C. Anal. ($C_{14}H_{20}BrN_3O_2$) C, H, N.

5-Bromo-2-[1-(2-methylbutyl)]-1,2,3,4-tetrahydro-7-nitro-8-isoquinolinamine (24f). 23f (2.6 g, 7.7 mmol) was nitrated as described previously (2.0 g, 69%).

1,4,7,8,9,10-Hexahydro-9-methylpyrido[3,4-*f*]quinoxaline-2,3-dione (25a). 24a (1.0 g, 3.49 mmol) was suspended in MeOH (100 mL) and treated with 20% Pd on carbon (0.2 g). The reaction vessel was purged with hydrogen gas and stirred for 4 h. Standard workup gave 1,2,3,4-tetrahydro-2-methyl-7,8-isoquinolinediamine as a brown solid (0.81 g, 90%). The diamine (0.7 g, 2.71 mmol) was condensed with oxalic acid using general procedure G to give product (0.36 g, 49%): mp >327 °C. Anal. ($C_{12}H_{13}N_3O_2{\cdot}HCl{\cdot}0.4H_2O$) C, H, N; Cl: calcd, 12.89; found, 11.09.

Alternatively, a mixture of **26a** (1.25 g) and 20% Pd/C (0.5 g) in 100 mL of DMF was shaken on a Parr apparatus under 52 psi of H_2(g) for 15.5 h. After filtration of the catalyst, the solvent was removed and the residue washed with ether and ether:acetone. The tan solid was dissolved in a minimum of water and neutralized with 5 drops of NH_4OH which induced slow precipitation. The solid was again collected and washed with water:acetone to give the product (0.65 g, 70%).

1,4,7,8,9,10-Hexahydro-9-ethylpyrido[3,4-*f*]quinoxaline-2,3-dione (25b). In a manner similar to that described for **25a, 24b** (1.00 g, 3.33 mmol) was reduced to give 1,2,3,4-tetrahydro-2-ethyl-7,8-isoquinolinediamine (0.91 g, 100%) and then condensed with oxalic acid to give the product as the HCl salt: mp 313–315 °C. Anal. ($C_{13}H_{15}N_3O_2{\cdot}HCl{\cdot}0.2H_2O$) C, H, N; Cl: calcd, 12.42; found, 11.95.

1,4,7,8,9,10-Hexahydro-9-(1-methylethyl)pyrido[3,4-*f*]-quinoxaline-2,3-dione (25c). In a similar manner as described above 1,2,3,4-tetrahydro-2-(1-methylethyl)-7,8-isoquinolinediamine was prepared from **24c** and condensed with oxalic acid using general procedure G to give product as the HCl salt.

1,4,7,8,9,10-Hexahydro-9-(1-methylpropyl)pyrido[3,4-*f*]quinoxaline-2,3-dione (25d). The HCl salt of **24d** was reduced using general procedure F to give 1,2,3,4-tetrahydro-2-(1-methylpropyl)-7,8-isoquinolinediamine, which was condensed with oxalic acid to give product after neutralizing with NH_4OH.

1,4,7,8,9,10-Hexahydro-9-[1-(3-methylbutyl)pyrido[3,4-*f*]quinoxaline-2,3-dione (25e). In a similar manner as described above, 1,2,3,4-tetrahydro-2-(3-methylbutyl)-7,8-isoquinolinediamine was prepared from **24e** and condensed with oxalic acid using general procedure G to give product as the HCl salt: mp 316–318 °C. Anal. ($C_{16}H_{21}N_3O_2{\cdot}HCl{\cdot}1.1H_2O$) C, N; H: calcd, 7.10; found, 6.66.

1,4,7,8,9,10-Hexahydro-9-[1-(2-methylbutyl)]pyrido[3,4-*f*]quinoxaline-2,3-dione (25f). 24f (2.0 g) was hydrolyzed as previously described to yield the aniline (1.78 g, 90%). The nitroaniline (1.78 g) was reduced as previously described to give the diamine, which was used directly in the condensation with oxalic acid as previously described (1.0 g, 80%). The methanesulfonate salt was made: mp 170–172 °C. Anal. ($C_{16}H_{21}N_3O_2{\cdot}1.3\ CH_4O_3S{\cdot}0.5H_2O$) C, H, N.

6-Bromo-1,4,7,8,9,10-hexahydro-9-methylpyrido[3,4-*f*]-quinoxaline-2,3-dione Methanesulfonate (26a). 24a (1.02 g, 3.49 mmol) was reduced according to general procedure E to give 5-bromo-1,2,3,4-tetrahydro-2-methyl-7,8-isoquinolinediamine as a tan oil and then condensed with oxalic acid in methanesulfonic acid to give the product as the methanesulfonic acid salt: mp >310 °C. Anal. ($C_{12}H_{12}BrN_3O_2{\cdot}CH_4O_3S$) C, H, N, Br.

6-Bromo-1,4,7,8,9,10-hexahydro-9-ethylpyrido[3,4-*f*]quinoxaline-2,3-dione (26b). 24b was reduced according to general procedure E to give 5-bromo-1,2,3,4-tetrahydro-2-ethyl-7,8-isoquinolinediamine, which was then condensed with oxalic acid in methanesulfonic acid to give the product as the methanesulfonic acid salt: mp >301 °C. Anal. ($C_{13}H_{14}BrN_3O_2{\cdot}CH_4O_3S$) C, H, N, Br.

6-Bromo-1,4,7,8,9,10-hexahydro-9-[1-(1-methylpropyl)]-pyrido[3,4-*f*]quinoxaline-2,3-dione Methanesulfonate (26c). 24d was reduced according to general procedure E to give 5-bromo-1,2,3,4-tetrahydro-2-(1-methylpropyl)-7,8-isoquinolinediamine, which was then condensed with oxalic acid in methanesulfonic acid to give the product as the methane-

sulfonic acid salt: mp >316 °C. Anal. ($C_{15}H_{18}BrN_3O_2 \cdot CH_4O_3S$) C, H, N, Br, S.

6-Bromo-1,4,7,8,9,10-hexahydro-9-[1-(3-methylbutyl)]-pyrido[3,4-*f*]quinoxaline-2,3-dione Methanesulfonate (26d). **24e** was reduced according to general procedure E to give 5-bromo-1,2,3,4-tetrahydro-2-(3-methylbutyl)-7,8-isoquinolinediamine, which was then condensed with oxalic acid in methanesulfonic acid to give the product as the methanesulfonic acid salt: mp >305 °C. Anal. ($C_{16}H_{20}BrN_3O_2 \cdot CH_4O_3S$) C, H, N, Br, S.

6-Bromo-1,4,7,8,9,10-hexahydro-9-[1-(2-methylbutyl)]-pyrido[3,4-*f*]quinoxaline-2,3-dione Methanesulfonate (26e). **24f** was hydrolyzed, and the nitroaniline (0.33 g) was reduced with Raney nickel as previously described. The resulting diamine was condensed with oxalic acid as previously described and converted into the methanesulfonate salt: Yield 0.20 g, 43%; mp 298–302 °C. Anal. ($C_{16}H_{20}BrN_3O_2 \cdot CH_4O_3S$) C, H, N.

General Procedure K. 1,4,7,8,9,10-Hexahydro-9-methyl-6-nitropyrido[3,4-*f*]quinoxaline-2,3-dione (27a). A solution of **25a** (0.64 g, 2.77 mmol) in concentrated sulfuric acid (20 mL) was treated with a solution of potassium nitrate (0.29 g, 2.87 mmol) in 2 mL of concentrated sulfuric acid. After stirring overnight at room temperature the reaction mixture was poured onto ice, cooled in a dry ice bath, and then basified with NH_4OH to pH 8.9. The orange/yellow solid was collected by filtration, washed consecutively with water, acetone, and ether, and dried to give 0.78 g (100% yield).

1,4,7,8,9,10-Hexahydro-9-methyl-6-nitropyrido[3,4-*f*]-quinoxaline-2,3-dione Methanesulfonate. 27a (1.58 g, 5.723 mmol) was dissolved in DMF (500 mL), 3 N $MeSO_3H$ was added, and the reaction was stirred overnight. The solvent was removed and washed with ether and acetone, and the light yellow solid was dried over P_2O_5 *in vacuo* to give product as the methanesulfonic acid salt (1.45 g, 875): mp >300 °C. Anal. ($C_{12}H_{12}N_4O_4 \cdot 1.1\ CH_4O_3S \cdot 0.63H_2O$) C, H, N, S.

9-Ethyl-1,4,7,8,9,10-hexahydro-6-nitropyrido[3,4-*f*]-quinoxaline-2,3-dione Methanesulfonate (27b). 25b (0.61 g, 2.16 mmol) was nitrated in concentrated sulfuric acid (20 mL) upon treatment with a solution of potassium nitrate (0.25 g, 2.47 mmol) in 2 mL of concentrated sulfuric acid. After stirring for 3 h, the reaction mixture was poured onto ice and then cooled in an isopropyl alcohol/dry ice bath. The pH was adjusted to 8.9 with NH_4OH, and the yellow solid collected by filtration (0.59 g, 94%). The product was suspended in DMF (300 mL) and stirred with the addition of 3 N methanesulfonic acid (1 mL). The color changed from orange to yellow, and the solid dissolved. Solvent was removed *in vacuo*, and the residue was washed with acetone and dried (0.73 g, 92%): mp 302–304 °C. Anal. ($C_{13}H_{14}N_4O_4 \cdot 1.07\ CH_3SO_3H \cdot 0.25H_2O$) C, H, N, S.

9-(1-Methylethyl)-1,4,7,8,9,10-hexahydro-6-nitropyrido-[3,4-*f*]quinoxaline-2,3-dione Methanesulfonate (27c). 25c (0.54 g, 1.83 mmol) was nitrated according to general procedure K and converted to the methanesulfonate salt as previously described (0.36 g, 50% (2 steps)): mp 318–321 °C. Anal. ($C_{14}H_{16}N_4O_4 \cdot CH_4O_3S \cdot 2H_2O$) C, H, N, S.

9-(1-Methylpropyl)-1,4,7,8,9,10-hexahydro-6-nitropyrido-[3,4-*f*]quinoxaline-2,3-dione Methanesulfonate (27d). 25d (0.94 g, 2.90 mmol) was changed to the free amine (0.84 g, 100%) and then nitrated according to general procedure K. That product was converted to the methanesulfonate salt as described previously (0.90 g, 79%): mp 290–292 °C dec. Anal. ($C_{15}H_{18}N_4O_4 \cdot CH_4O_3S \cdot 1.3H_2O$) C, N, S; H: calcd, 5.66; found, 5.06.

9-[1-(3-Methylbutyl)]-1,4,7,8,9,10-hexahydro-6-nitropyrido[3,4-*f*]quinoxaline-2,3-dione Methanesulfonate (27e). 25e (0.94 g, 2.90 mmol) was dissolved in boiling water and basified with NH_4OH (drops) to give the free amine (0.84 g, 100%). This was then nitrated according to general procedure K and converted to the methanesulfonate salt as described previously (0.90 g, 79%): mp 257–259 °C dec. Anal. ($C_{16}H_{20}N_4O_4 \cdot CH_4O_3S$) C, H, N, S.

9-[1-(2-Methylbutyl)]-1,4,7,8,9,10-hexahydro-6-nitropyrido[3,4-*f*]quinoxaline-2,3-dione Hydrochloride (27f). **25f** was nitrated as previously described: mp 283–285 °C. Anal. ($C_{16}H_{20}N_4O_4 \cdot HCl$) C, H, N.

6-Amino-1,4,7,8,9,10-hexahydro-9-methylpyrido[3,4-*f*]-quinoxaline-2,3-dione (28). A solution **27a** (0.77 g, 2.79 mmol) in 100 mL of AcOH was treated with 5% Pd/C (0.1 g) and shaken on a Parr apparatus at 52 psi for 9.5 h. After removing the catalyst by filtration, the solvent was removed and the residue recrystallized from water to give the mono-AcOH salt (0.14 g). The filtrate was basified to pH 9 with NH_4OH to precipitate the free base, which was further washed with water and then ether to give a brown solid (0.46 g): mp 291–294 °C. Anal. ($C_{12}H_{14}N_4O_2 \cdot 2H_2O$) C, H, N.

***N*-(1,2,3,4,7,8,9,10-Octahydro-9-methyl-2,3-dioxopyrido-[3,4-*f*]quinoxalin-6-yl)acetamide (29).** A mixture of **28** (0.27 g, 1.1 mmol) in 25 mL of AcOH and 10 mL of acetic anhydride was stirred at room temperature for 18 h. After removing the solvent, the residue was washed with ether, dissolved in water, and then basified with saturated $NaHCO_3$. The flask was cooled in the freezer for 2 h and the precipitate collected by filtration, washed consecutively with water and ether, and dried over P_2O_5 *in vacuo* to give the acetamide (0.2 g, 63%): mp >300 °C. Anal. ($C_{14}H_{16}N_4O_3 \cdot 0.5H_2O$) C, H, N.

1,2,3,4,7,8,9,10-Octahydro-9-methyl-2,3-dioxopyrido-[3,4-*f*]quinoxaline-6-carbonitrile Methanesulfonate (30). A solution of **28** (0.53 g, 2.15 mmol) in H_2SO_4 (3 mL) was cooled to 0 °C and treated with $NaNO_2$ (0.16 g, 2.32 mmol) in 10 mL of H_2O. After 30 min, $NaHCO_3$ was added to adjust the pH of the reaction to 7. $K_2Ni(CN)_4$ was prepared by adding KCN (1.66 g, 25.5 mmol) in 4 mL of water to $NiSO_4 \cdot 6H_2O$ (1.43 g, 5.44 mmol) in 4 mL of water. This solution was added to the diazonium solution, stirred for 30 min at 0 °C, and then heated to near reflux for 30 min. The solid precipitate was filtered, washed with water:ether, and air-dried to give the product (0.24 g, 44%). The methanesulfonic acid salt was prepared as before. Anal. ($C_{13}H_{12}N_4O_2 \cdot CH_4O_3S$) Calcd: C, 47.72; H, 4.58; N, 15.90. Found: C, 20.33; H, 3.13; N, 5.63. IR (cm^{-1}) 2227.9 (CN); MS (CI, NH_3 in CH_4) *m/e* 257 (M + 1); HPLC 94%, C18 column, 10% MeOH/90% 0.05 M NaH_2PO_4, pH = 7.

Oxo(8-quinolinylamino)acetic Acid Ethyl Ester (32). A mixture of 8-aminoquinoline (14.4 g, 0.1 mol), ethyloxalyl chloride (16.4 g, 0.12 mol), and triethylamine (15 g, 0.15 mol) in 150 mL of $CHCl_3$ was stirred at room temperature for 18 h. The mixture was washed with water and extracted with methylene chloride. The combined organic layers were washed with saturated NaCl solution, dried over Na_2SO4, filtered, and evaporated. The residue was then crystallized from toluene: heptane to give the product (20.6 g, 86% yield). Anal. ($C_{13}H_{12}N_2O_3$) H, N; C: calcd, 63.93; found, 63.20.

[(5-Bromo-8-quinolinyl)amino]oxoacetic Acid Ethyl Ester (33). A solution of **32** (20 g, 82 mmol) in 200 mL of AcOH was treated with a solution of bromine (15.7 g, 0.198 mol) in 50 mL of AcOH and stirred at room temperature for 1 h. The solid was collected by filtration and washed with ether to give the product (23.5 g, 89%).

[(5-Bromo-7-nitro-8-quinolinyl)amino]oxoacetic Acid Ethyl Ester (34). A solution of **33** (23.5 g, 58 mmol) in 30 mL of fuming nitric acid and 150 mL of trifluoroacetic acid was heated at 80 °C for 18 h. The solvent was removed, and the residue was treated with water to give a solid. The solid was collected by filtration, washed with ether, and dried to give the nitro derivative (9.5 g, 45% yield).

1,4,7,8,9,10-Hexahydropyrido[2,3-*f*]quinoxaline-2,3-dione (35). A solution of **34** (1 g, 2.7 mmol) in 250 mL of AcOH was treated with 20% Pd on carbon (0.1 g) and shaken on a Parr apparatus under 52 psi of hydrogen gas for 21 h. After removal of the catalyst, the solvent was evaporated but gave negligible material. The catalyst/Celite was washed two times with 1 N HCl, and the aqueous solution was basified with $NaHCO_3$. The precipitate was collected by filtration, dried, and then recrystallized from MeOH/DMF to give product (0.26 g, 44% yield): mp >300 °C. Anal. ($C_{11}H_{11}N_3O_2 \cdot 1.5H_2O$) C, H, N.

[(6-Nitro-5-quinolinyl)amino]oxoacetic Acid Ethyl Ester (37). A solution of 5-amino-6-nitroquinoline (3 g, 16 mmol) in DMF (50 mL) was treated with triethylamine (3.2 g, 32 mmol) and ethyloxalyl chloride (3.2 g, 24 mmol) and heated

at 50 °C for 1 h. After removing the triethylamine hydrochloride by filtration, the filtrate was concentrated and diethyl ether added. The resulting solid was collected by filtration (3.7 g, 80%).

1,4,7,8,9,10-Hexahydropyrido[3,2-*f*]quinoxaline-2,3-dione (38). A solution of **37** (3.7 g) in AcOH (100 mL) was shaken with PtO_2 under a hydrogen atmosphere (51 psi) on a Parr apparatus for 15.5 h. Additional PtO_2 (0.15 g) was added and the apparatus recharged with hydrogen and shaken for 32 h. After standard workup, the syrupy residue was heated in MeOH:ether to give a solid. Recrystallization from MeOH, DMF, and water gave a light, yellow solid: mp >285 °C. Anal. ($C_{11}H_{11}N_3O_2 \cdot 0.5H_2O$) C, H, N.

[(1,2-Dihydro-6-nitro-5-quinolinyl)amino]oxoacetic Acid Ethyl Ester (39). A solution of **37** (5 g, 17.5 mmol) in AcOH (80 mL) was treated with $NaCNBH_3$ (2.2 g, 35 mmol) portionwise under an argon atmosphere and stirred for 4 h. Water (100 mL) was added to the reaction mixture, and the resulting solid was collected by filtration and dried (3.9 g, 78%).

[(1,2,3,4-Tetrahydro-6-nitro-5-quinolinyl)amino]oxoacetic Acid Ethyl Ester (40). A solution of **39** (4.8 g, 16.5 mmol) in THF (250 mL) was treated with triphenylphosphine (5 g) and rhodium trichloride (0.83 g) and shaken in a Parr apparatus under a hydrogen atmosphere (54 psi) for 40 min. The solvent was evaporated, and the residue was purified by silica gel chromatography (4:1 to 1:1 heptane:EtOAc) to give the a red solid (4.22 g, 87%).

[(8-Bromo-1,2,3,4-tetrahydro-6-nitro-5-quinolinyl)amino]oxoacetic Acid Ethyl Ester (41). A mixture of **40** (0.15 g, 0.5 mmol) and *N*-bromosuccinimide (0.23 g, 1 mmol) in DMF (2 mL) was stirred at room temperature for 18 h. The solution was poured onto CH_2Cl_2:water and separated and the water layer back-extracted with CH_2Cl_2. The combined organic layers were washed with water, dried over Na_2SO4, filtered, and evaporated. The residue was purified by silica gel chromatography (4:1 to 1:1 heptane:EtOAc) to give the product (0.15 g, 81%).

6-Bromo-1,4,7,8,9,10-hexahydropyrido[3,2-*f*]quinoxaline-2,3-dione (42). Raney nickel was washed with acetone and then with THF. A solution of **41** (0.3 g, 0.8 mmol) in THF (30 mL) was added to the Raney nickel, and the mixture was stirred at room temperature under a hydrogen atmosphere for 2 h. The catalyst was removed, washed with MeOH, and filtered, and the combine filtrates were evaporated to give a yellow solid (0.1 g, 42% yield). HPLC analysis showed that 7% of the product was the debrominated quinoxalinedione.

5,6-Quinolinediamine (43). A solution of 5-amino-6-nitroquinoline (25 g, 0.13 mol) in THF (500 mL) and MeOH (500 mL) was shaken with Raney nickel (7 g) under a hydrogen atmosphere (50.4 psi) on a Parr apparatus for 3.4 h. Standard workup gave the product.

1,4-Dihydropyrido[3,2-*f*]quinoxaline-2,3-dione (44). 43 (11.3 g, 71 mmol) was condensed with oxalic acid according to general procedure G to give a solid (12.3 g, 83%). Anal. ($C_{11}H_7N_3O_2 \cdot 2H_2O$) C, N; H: calcd, 4.43; found, 3.27.

1,2,3,4-Tetrahydro-7-methyl-2,3-dioxopyrido[3,2-*f*]quinoxalin-7-ium Methanesulfonate (45). 44 (5 g, 23 mmol) and dimethyl sulfate (7.2 g) were dissolved in DMF (40 mL) and heated at 120 °C for 20 h. The solvent was removed *in vacuo*, the residue triturated with MeOH, and the precipitate collected by filtration (6 g).

1,4,7,8,9,10-Hexahydro-7-methylpyrido[3,2-*f*]quinoxaline-2,3-dione (46). A mixture of **45** (6 g, 18 mmol) and PtO_2 (0.5 g) in MeOH (100 mL) was shaken on a Parr apparatus under a hydrogen atmosphere (52 psi) for 7.2 h. After removal of the catalyst and evaporation, the residue was triturated with water. The precipitate was collected by filtration and recrystallized from DMF and water (1.7 g). Anal. ($C_{12}H_{13}N_3O_2 \cdot 0.18H_2O$) C, H, N.

***N*-(1,2,3,4-Tetrahydro-5-quinolinyl)acetamide (48).** A solution of 5-nitroquinoline (50 g, 0.29 mol) and acetic anhydride (30 mL) in AcOH (1 L) was shaken with 5% Pd on carbon (3 g) under a hydrogen atmosphere (50 psi) for 10.4 h. The mixture was then treated with PtO_2 (1 g), recharged with hydrogen, and shaken an additional 2 h. A standard workup and recrystallization from EtOAc gave the product (23 g, 42%).

***N*-[1,2,3,4-Tetrahydro-1-(1-oxobutyl)-5-quinolinyl]acetamide (49).** A mixture of **48** (10 g, 53 mmol), butyric anhydride (12.5 g, 79 mmol), and triethylamine (10 g, 0.1 mmol) in CH_2Cl_2 (50 mL) was heated at reflux for 4 h. The solution was washed with water, dried over Na_2SO_4, filtered, and evaporated. The residue was chromatographed on silica gel (40–60% EtOAc in heptane) to give the product (9.5 g, 69%).

***N*-[1,2,3,4-Tetrahydro-6-nitro-1-(1-oxobutyl)-5-quinolinyl]acetamide (50).** A solution of **49** (7.3 g, 28 mmol) in AcOH (50 mL) was treated with fuming nitric acid (10 mL) and stirred for 1 h. After removal of the solvent, an aqueous solution of potassium carbonate was added, and the mixture was extracted with CH_2Cl_2. The organic residue was chromatographed on silica gel (40% EtOAc in heptane to EtOAc) to give **43** (4.5 g), a mixture of the 6- and 8-nitro adducts (2.2 g), and the 8-nitro derivative (1 g).

1,2,3,4-Tetrahydro-6-nitro-5-quinolinamine (51). 50 (2.1 g, 6.9 mmol) was hydrolyzed in refluxing 2 N HCl. After cooling, the solution was basified with aqueous NaOH and extracted with CH_2Cl_2 to give an orange solid (1.3 g, 97%).

Ethyl 6-[(2-Ethoxy-1,2-oxoethyl)amino]-3,4-dihydro-6-nitro-α-oxo-1(2*H*)-quinolineacetate (52). A mixture of **51** (1.29 g, 6.7 mmol), ethyl oxalyl chloride (2.7 g, 20 mmol), and triethylamine (2.7 g, 27 mmol) in THF (40 mL) was stirred for 18 h. The solvent was removed and the residue partitioned between CH_2Cl_2 and water. The organic residue was chromatographed on silica gel (1:1 EtOAc:heptane) to give a syrup (2.8 g, 85%).

Ethyl 1,2,3,4,9,10-Hexahydro-α,2,3-trioxopyrido[3,2-*f*]quinoxaline-7(8*H*)-acetate (53). A solution of **54** (2.9 g) in EtOH was shaken with 5% Pd on carbon (1 g) under a hydrogen atmosphere (50 psi) on a Parr apparatus for 40 min. After a standard workup, the residue was chromatographed on silica gel (10–50% EtOAc in heptane) and then recrystallized from hot MeOH and toluene to give a solid (0.3 g, 14%): mp >285 °C. Anal. ($C_{15}H_{15}N_3O_5 \cdot 0.7H_2O$) C, H; N: calcd, 12.74; found, 13.16.

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Supporting Information Available: Coordinates for two of the structures (**1** and **27a**) from the pharmacophore model, in SYBYL .MOL2 file format (6 pages). Ordering information is given on any masthead page.

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(1) Portions of this work were presented at the 24th Annual Meeting of the Society for Neurosciences, Miami Beach, FL, November 1994, Abst. 96.3, and 1st Winter Conference on Bioorganic and Medicinal Chemistry in Steamboat Springs, CO, February 1995. In honor of Koji Nakanishi's 70th birthday.

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