Brief Articles

Design and Synthesis of Novel Quinoxaline-2,3-dione AMPA/Gly_N Receptor Antagonists: Amino Acid Derivatives

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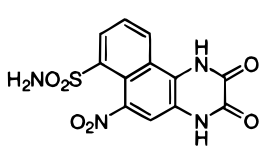
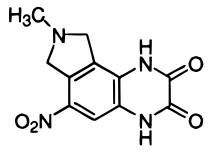
PNQX (1,4,7,8,9,10-hexahydro-9-methyl-6-nitropyrido[3,4-*f*]quinoxaline-2,3-dione) is a potent AMPA (IC_{50} = 0.063 μM) and Gly_N (IC_{50} = 0.37 μM) receptor antagonist that was developed in our laboratories. While possessing a desirable in vitro and in vivo activity profile, this compound suffers from low aqueous solubility. In an effort to improve its potency and physical properties, we have designed and synthesized novel ring-opened analogues **4**, **6**, **9**, and **11**. Modeling analyses demonstrated that, while the 5-substituent in these analogues was forced to adopt an out-of-plane conformation due to steric contacts with neighboring substituents, the overall structure retained a good fit to a previously described AMPA pharmacophore model. This nonplanar orientation may lessen efficient packing in the solid state, compared to PNQX, leading to increased water solubility. Indeed, several nonplanar analogues containing appropriate functionalities, for example, the sarcosine analogue **9**, were found to retain AMPA (IC_{50} = 0.14 μM) and Gly_N (IC_{50} = 0.47 μM) receptor affinity and possess improved aqueous solubility compared to PNQX. The synthesis and the SAR of these compounds are discussed.

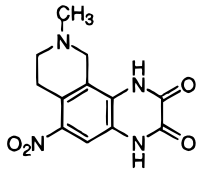
Introduction

Glutamic acid is the principal excitatory neurotransmitter in the central nervous system (CNS), and it plays a significant role in regulating neuronal activity via various glutamate receptors. Excessive release of glutamic acid can cause overexcitation of these receptors, leading to neuronal death. Within the ionotropic class of these receptors, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), kainate (KA), and *N*-methyl-D-aspartate (NMDA) receptors have received significant attention due to their implication in a variety of pathophysiological conditions such as global and focal ischemia, epilepsy, and Parkinson's, Huntington's, and Alzheimer's diseases.[1−9] We have focused on developing potent antagonists for the AMPA, KA, and NMDA associated glycine (Gly_N) receptors.

A number of competitive AMPA receptor antagonists have been developed in the past few years, several of which have shown neuroprotective activity. Examples include NBQX,[10] NS 257,[11] YM 90K,[12] and PNQX.[13] Of these, NBQX has been studied extensively due to its selective affinity for the AMPA receptor.[10] The focus of this study was PNQX, which displays high affinity for both the AMPA and Gly_N receptors.[13] PNQX is also potent in the maximal electroshock assay (MES; ED_{50} = 0.44 mg/kg, iv), which has been used as a measure of anticonvulsant activity and a preliminary in vivo indicator of CNS penetration. Potency is maintained in animal stroke models, such as middle carotid artery occlusion (MCAO) and global forebrain ischemia.[14] Due to its excellent in vitro and in vivo profile, PNQX was selected as a lead compound in our stroke program. The major disadvantage of PNQX is its low aqueous solubility (0.0086 mg/mL, Table 2), which is also observed in several other excitatory amino acid antagonists containing a quinoxaline-2,3-dione motif. The poor aqueous solubility leads to the potential of crystallization in the kidney. Other disadvantages are the short duration of action following iv administration (<5 min) in the MES assay and a lengthy linear chemical synthesis, which precludes rapid analoging. Thus, our primary goal in the identification of potential backups for PNQX was to design and synthesize equipotent molecules with improved aqueous solubility.







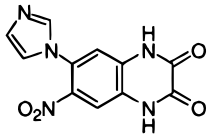


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Chart 1. Nonplanar Analogues of PNQX

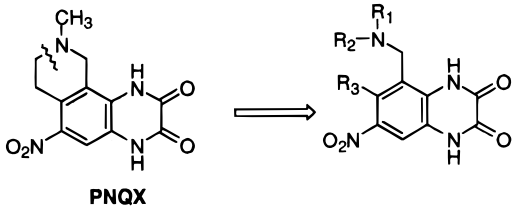


Chart 2. Pharmacophore Model for AMPA Receptor Antagonists

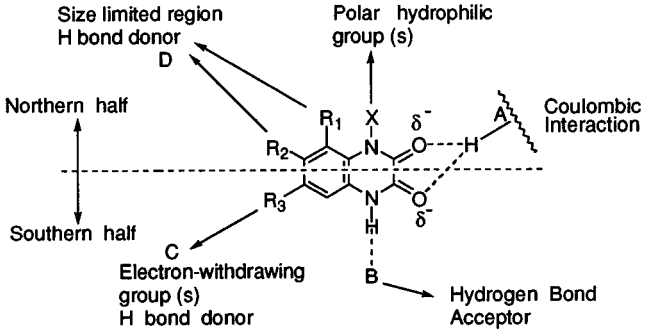
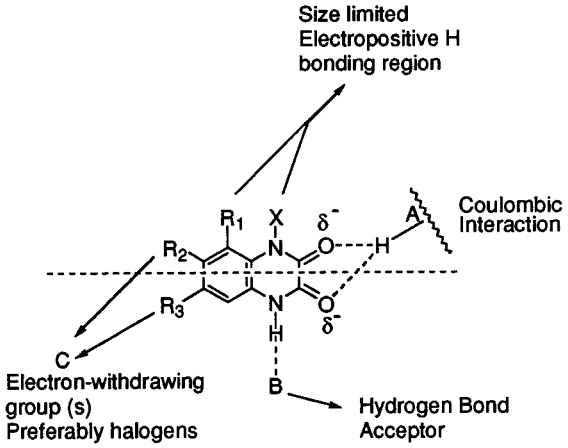


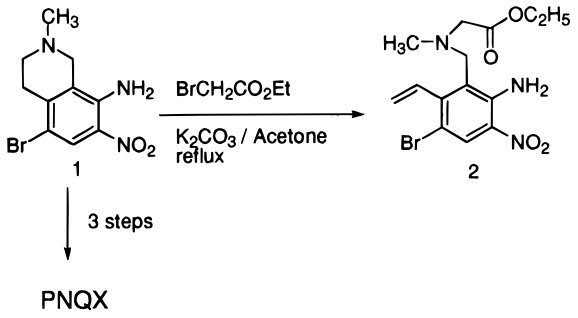
Chart 3. Pharmacophore Model for Gly_N Receptor Antagonists



The poor solubility of PNQX is due in part to the relatively planar tricyclic motif of the molecule, which is likely responsible for the efficient packing in the solid state, which can in turn decrease solubility. Thus, our initial strategy to make soluble PNQX analogues was to open the reduced pyridine ring (Chart 1) to give nonplanar, C-5- and C-6-substituted quinoxaline-2,3-dione derivatives with a substituted aminomethyl side chain at C-5. Polar substituents, such as a carboxymethyl moiety, could then be added onto the amine functionality to further improve the aqueous solubility of the final compounds.

To preserve affinity at the AMPA/Gly_N receptors, the design of new analogues was also based on AMPA/Gly_N pharmacophore models (Charts 2 and 3). The AMPA pharmacophore model was generated by superimposing several PNQX analogues onto other active AMPA antagonists, and it has been described earlier by Bigge et al.[8,13] It was obvious from this model that the 7-nitroquinoxaline-2,3-dione motif was very important for good AMPA/Gly_N receptor affinity and that the N-4, C-5, and C-6 positions were amenable for substitution with groups of limited polarity and bulk. For design purposes,

Scheme 1. Hofmann Degradation of the Isoquinoline Intermediate



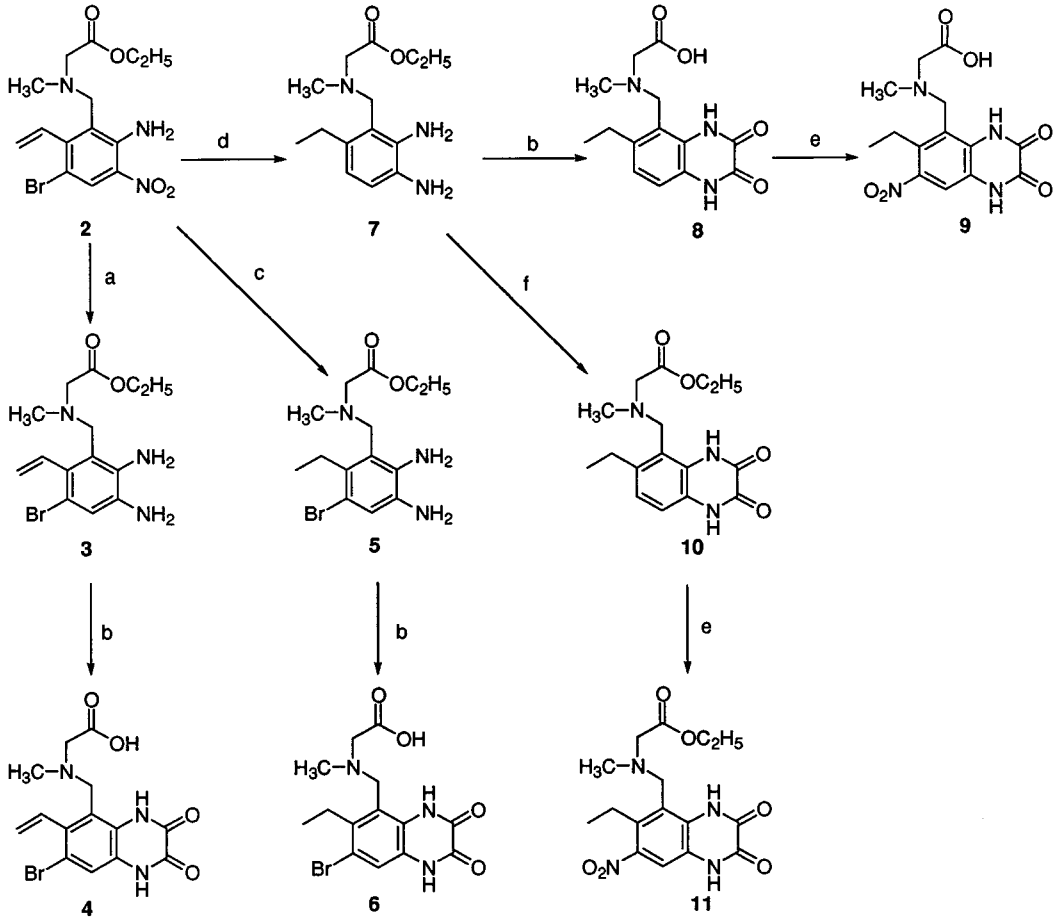
we simplified the model by dividing PNQX into halves: viz. the northern half and the southern half. On the basis of the SAR of PNQX and its analogues, we decided to keep the southern portion intact and make necessary modifications in the northern portion, particularly the C-5 and C-6 positions, to improve the physical properties of the new quinoxaline-2,3-dione derivatives. During the course of writing this manuscript, new 5-(aminomethyl)-quinoxaline-2,3-dione analogues were reported.[15] However, these compounds are unsubstituted at C-6, and the overall rationale and synthesis differ significantly from this report.

Chemistry

Synthesis of new analogues was designed based on the intermediates available from the PNQX synthesis. Thus, the synthetic strategy (Scheme 1) involved Hofmann degradation of the substituted tetrahydroisoquinoline intermediate **1** with ethyl bromoacetate in the presence of potassium carbonate in boiling acetone. The reaction gave a good yield of the desired benzylamine derivative, [(2-amino-5-bromo-3-nitro-6-vinylbenzyl)methylamino]acetic acid ethyl ester (**2**), and has proven to be an excellent route to multifunctional phenyl rings. Compound **2** was the critical intermediate for the synthesis of the new acyclic analogues of PNQX, as it was appropriately substituted with 3-amino-2-nitro groups necessary for conversion to the corresponding quinoxaline-2,3-dione derivatives.

The benzylamine derivative **2** was then selectively reduced using catalytic hydrogenation to give the corresponding *o*-phenylenediamine derivatives (Scheme 2). Reduction of **2** in the presence of RaNi in THF gave the 6-vinyl-7-bromo-*o*-phenylenediamine derivative **3**, whereas the use of ethanol as a solvent in the reduction step gave the corresponding 6-ethyl-7-bromo derivative **5**. Replacing RaNi with Pd/C (20%) as the catalyst and methanol as a solvent gave the desbromo-6-ethyl-*o*-phenylenediamine derivative **7**. The *o*-phenylenediamine derivatives **3**, **5**, and **7**, on treatment with oxalic acid in 2 N HCl at 90 °C, gave the corresponding quinoxaline-2,3-dione derivatives **4**, **6**, and **8**, respectively. The ethyl sarcosinate analogue **11** was synthesized by cyclizing the intermediate **7** with dimethyl oxalate in boiling THF to give the corresponding quinoxaline-2,3-dione derivative **10**. The quinoxaline-2,3-dione derivative **10** was subsequently nitrated with KNO_3 in sulfuric acid at 0 °C to give the 7-nitro derivative **11**.

Scheme 2. Synthesis of 5-Aminomethyl Derivatives of Quinoxaline-2,3-diones[a]



[a] (a) RaNi/H_2/THF; (b) (CO_2/H)$_2$/Cl; (c) RaNi/H_2/EtOH; (d) Pd/C (20%), H_2/EtOH; (e) KNO_3/H_2SO_4; (f) (CO_2Me)$_2$/EtOH.

Table 1. Acyclic PNQX Analogues: Sarcosine Derivatives

	IC_{50} (μM)[a]	
compd	AMPA	Gly_N
PNQX	0.063	0.37
4	1.18 (±0.29)	NT
6	2.32 (±0.63)	NT
8	>10	>10
9	0.14 (±0.02)	0.47
11	1.59 (±1.17)	>10

[a] NT, not tested. The AMPA and Gly_N data are the mean of three experiments with triplicate incubations in each experiment. SEM for compound **9** in [^{3}H]Gly_N assay (n = 3) is ±0.007.

Results and Discussion

The in vitro binding data for the sarcosine derivatives are summarized in Table 1. The nonplanar acyclic analogue, sarcosine derivative **9**, retained the AMPA and Gly_N receptor binding affinity shown by PNQX, suggesting that the northern portion of the molecule is indeed amenable to modifications and can tolerate bigger groups than that found on PNQX. Modeling studies confirmed that the aminomethyl side chain is held nearly 90° out of plane of the quinoxaline-2,3-dione ring system (Figure 1) due to steric contacts with neighboring groups. Modeling also showed that one oxygen of the carboxylic acid group, as well as the amine from the aminomethyl side chain, can interact at the same H-bond donor site (Chart 2, site D) of the receptor in the AMPA pharmacophore model as the piperidine ring nitrogen of PNQX, albeit from different directions. However, the lack of an enhancement in potency over

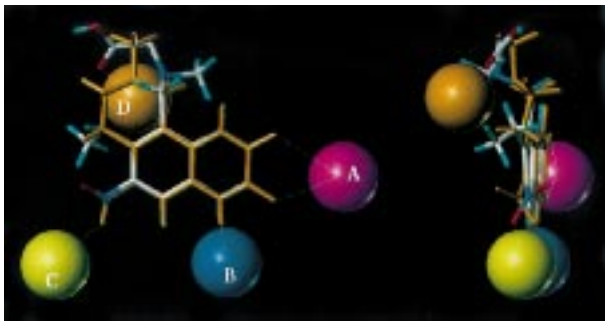


Figure 1. Orthogonal view of the overlay of sarcosine derivative **9** (colored by atom type) and PNQX (orange) fit to an AMPA pharmacophore model.[8,13] Four receptor interaction site points (A−D) from the model have been included. Note the relatively nonplanar orientation of the amino acid side chain, particularly the *N*-methyl group.

PNQX suggests that an additional hydrogen bond to this site contributes little to overall binding energy. The overall SAR of the new sarcosine analogues indicates that the southern portion of the molecule is important for activity, with the amide carbonyl groups at C-2 and C-3 participating in a Coulombic interaction with a positively charged site (Chart 2, site A) at the receptor. The electron-withdrawing nitro group at C-7 not only interacts at a hydrogen bond-accepting site (Chart 3, site C) near the southwestern region of the molecule but also enhances the Coulombic interaction in the eastern portion by rendering the proton on the N-4 nitrogen

Table 2. Measured Solubility and Predicted pK_a's[b]

compd	solubility[a] (μg/mL)	predicted pK_a's[b]
PNQX	8.6	6.9, *7.4*, 10.6
4	170	2.3, *8.3*, 8.8, 11.7
6	140	2.3, *8.3*, 8.8, 11.9
9	420	2.3, *7.8*, 8.8, 10.7
11	150	*5.9*, 8.5, 10.6

[a] Solubility in pH 7.4, 50 mM phosphate buffer. [b] Predicted pK_a's using ACD Labs log *D* suite version 3.00.

more acidic. A nitro group seems optimum here, as the 7-bromo analogue **6** had much less affinity for the AMPA receptor and the analogue with no substitution at C-7 (**8**) was inactive. These observations are consistent with the primary sites of interaction indicated earlier in the AMPA pharmacophore model based on PNQX analogues.[8,9,13]

The sarcosine analogue **9** also shows excellent affinity for the Gly_N site, which indicates that the polar carboxylic acid group in the aminomethyl side chain has enough flexibility to interact with the H-bonding interaction site at the Gly_N receptor, which appears to be directed in the northeastern portion of the molecule in comparison to the northwestern orientation for the AMPA receptor. This northeastern orientation of the sarcosine side chain is favored due to the presence of the ethyl group at C-6. This ethyl substituent, in combination with the quinoxaline ring N-4−H, constrains the aminomethyl side chain at C-5 out of the plane of the quinoxaline ring system. The ethyl sarcosinate derivative **11** was synthesized to reduce overall polarity, since **9** was not active in the MES assay. This compound was much weaker at the AMPA receptor in comparison to the sarcosine derivative **9** but showed weak activity in the MES assay (20% protection @ 30 mg/kg, iv). This is consistent with the ester probably acting as a prodrug for the required carboxylic acid at the AMPA receptor.

The strategy of increasing solubility by making the nonplanar compounds was successful, as sarcosine derivatives **4**, **6**, **9**, and **11** were much more soluble than PNQX at pH 7.4 (Table 2). These results can be explained by examining the difference in ionization potential as well as the difference in rigidity and planarity of the PNQX and its acyclic analogues.

The aqueous solubility of PNQX is limited by the fact that it is predicted (pK_a's, Table 2) to exist as a zwitterion at pH 7.4. That is, half of the species in solution at pH 7.4 should be zwitterionic and half should be negatively charged. Thus, if the predicted pK_a's are accurate and we assume that the zwitterionic form is its least soluble form, the solubility of the zwitterionic form of PNQX is 4.3 μg/mL. This zwitterionic form is likely to be nearly insoluble because it sets up the formation of rigid and somewhat planar zwitterionic dimers that can then pack efficiently and/or utilize hydrogen bonding to form a robust crystal lattice. These dimers are planar and rigid due to the planarity and rigidity of the monomers and due to the charges being located at opposite ends of the monomers.

None of the analogues (**4**, **6**, **9**, and **11**) are as planar or rigid as PNQX. Although all but one is predicted to be primarily zwitterionic at pH 7.4, the solubilities of those zwitterions are much higher than that of PNQX (Table 2). This is likely due to the proximity of the charges as observed in amino acids and the fact that both the monomers and the zwitterionic dimers that can form are less planar and less rigid than those of PNQX. This would result in a reduction in packing efficiency in the solid state, leading to increased solubility. The solubility differences between the acyclic PNQX analogues themselves are less dramatic and are most likely driven by differences in their pK_a's since the most soluble of those analogues is also predicted to be most negatively charged at pH 7.4.

In conclusion, the strategy to synthesize new nonplanar acyclic analogues of PNQX has resulted in analogues such as the sarcosine derivative **9** that show comparable affinity for the AMPA receptor with at least 2-fold better affinity for the Gly_N receptor and improved solubility. Work is in progress to delineate other nonplanar analogues with better in vivo activity.

Experimental Section

[^{3}H]AMPA, [^{3}H]Glycine, and [^{3}H]Kainate Receptor Binding. Receptor binding was measured as percent displacement of [^{3}H]AMPA and [^{3}H]kainate from extensively washed rat cortical synaptosomal membranes. Using 50 mM Tri Hl buffer, pH 7.4, tubes containing membranes, 10 nM [^{3}H]-AMPA, 10 mM KSCN, and test compounds were incubated on ice for 60 min,[16] while other tubes containing 5 nM [^{3}H]-kainate, 20 mM $CaCl_2$, and test compounds were incubated on ice for 90 min.[17,18] Separately a 20 nM [^{3}H]glycine solution, in 50 mM Hepes KOH, pH 7.4, was incubated on ice for 30 min.[19] Nonspecific binding was determined by the presence of 1.0 mM glutamate. The incubation was terminated by rapid vacuum filtration through GF/B filters followed by 2 × 2-mL washes with appropriate ice-chilled buffer. Radioactivity was determined by liquid scintillation techniques, and IC_{50} values were determined by regression.

Maximal Electroshock Anticonvulsant Assay. Male CF-1 mice, 20−28 g, from Charles River Laboratories Portage, MI, were used in all experiments. All dose−response testing was conducted at the previously determined time of peak drug effect. Groups of 10 mice each were given various intravenous doses of antagonist dissolved in 5% dextrose in distilled water and administered into the retro-orbital sinus. Electroshock was delivered by corneal electrodes with 60-Hz alternating current (50 mA root-mean-squared for 0.2 s) using a constant-current stimulator (Wahlquist Instruments, Salt Lake City, UT). Untreated or vehicle-treated mice had reliable tonic extensor seizures with rearward extension of hind limbs for more than 3 s. Dose−effect experiments were analyzed by probit analysis.[20] These methods are similar to those published elsewhere.[21]

Solubility Measurements. Equilibrium solubilities of PNQX and its analogues (Table 2) were determined by adding an excess amount of compound to pH 7.4, 50 mM phosphate buffer followed by 5 min of sonification and overnight equilibration by moderate stirring at ambient temperature. Sample aliquots were removed and filtered through a 0.45-μm nylon filter. Solutions with known concentrations (standard solutions) were prepared for each compound to be used to quantify saturated solutions. Saturated and standard solutions were then analyzed by UV spectrophotometry with an HP 8452A diode array spectrophotometer using a quartz cell with a 1-cm path length. To keep the relationship between absorbance and concentration linear, some saturated solutions required dilution. The scanned wavelength range was 230−400 nm. Linear regression was performed on absorbance and concentration data of the standard solutions at an appropriately chosen wavelength, typically near 330 nm. This allowed the concentrations of the saturated solutions, i.e., solubilities, to be calculated at the corresponding wavelength.

Chemistry. All starting materials were purchased from Aldrich and used without additional purification. 5-Bromo-2-

methyl-7-nitro-1,2,3,4-tetrahydroisoquinolin-8-ylamine (**1**) was synthesized as previously described.[13] ^{1}H NMR spectra studies were recorded at 400 MHz in $CDCl_3$, DMSO-d_6, or CD_3OD. Chemical shifts are referenced to the residual proton signal for $CDCl_3$ (7.26 ppm), DMSO-d_6 (2.49 ppm), or CD_3OD (3.3 ppm).

Ethyl 2-{[(2-Amino-5-bromo-3-nitro-6-vinylphenyl)-methyl]methylamino}acetate (2). To a suspension of **1** (2.86 g, 10 mmol) and potassium carbonate (2.76 g, 20 mmol) in acetone (50 mL) was added bromoacetic acid ethyl ester (1.67 g, 10 mmol). The reaction mixture was stirred under reflux until TLC (SiO_2, petroleum ether:EtOAc, 1:1) indicated completion. Volatile materials were evaporated under vacuum, and water (150 mL) was added to the yellow residue. Product was extracted with EtOAc (2 × 150 mL), and the EtOAc extracts were washed with water (2 × 50 mL) and dried over $MgSO_4$. Product was purified by chromatography (SiO_2, petroleum ether:EtOAc, 95:5 to 75:25) to give 2.25 g (60%): mp 68−70 °C; ^{1}H NMR ($CDCl_3$) δ 1.29 (t, 3H, J = 7.2 Hz), 2.26 (s, 3H), 3.26 (s, 2H), 3.86 (s, 2H), 4.21 (q, 2H), 5.23 (dd, 1H, J = 1.2 Hz, J = 18 Hz), 5.69 (dd, 1H, J = 1.2 Hz, J = 11.5z), 6.57 (dd, 1H, J = 11.6 Hz, J = 18 Hz), 8.05 (bs, 2H), 8.36 (s, 1H); MS (CI) m/z 373 (M + H).

[(2,3-Diamino-5-bromo-6-vinylbenzyl)methylamino]-acetic Acid Ethyl Ester (3). A suspension of **2** (0.5 g, 1.34 mmol) and RaNi (0.5 g) in THF (75 mL) was hydrogenated (H_2, 50 psi) in a Parr apparatus. The reaction mixture was filtered, and the filtrate was evaporated to give 0.45 g of the diamine **3**, which was used without further purification: ^{1}H NMR ($CDCl_3$) δ 1.28 (t, 3H, J = 7.2 Hz), 2.27 (s, 3H), 3.22 (s, 2H), 3.79 (s, 2H), 4.03 (bs, 2H), 4.17 (bs, 4H), 4.19 (q, 2H), 5.14 (dd, 1H, J = 1.8 Hz, J = 16 Hz), 5.54 (dd, 1H, J = 1.8 Hz, J = 10 Hz), 6.59 (m, 1H), 6.93 (s, 1H).

[(7-Bromo-6-vinyl-2,3-dioxo-1,2,3,4-tetrahydroquinox-alin-5-ylmethyl)methylamino]acetic Acid Hydrochloride (4). A solution of **3** (0.45 g) and oxalic acid (0.327 g, 2.6 mmol) in aqueous 2 N HCl (20 mL) was heated to 80 °C. After 4 h, the reaction mixture was cooled and triturated with a spatula to give an off-white precipitate, which was filtered, dried, and recrystallized from a water:acetone mixture to give 0.162 g of **4** (31%): mp >300 °C; ^{1}H NMR (D_2O) δ 2.37 (s, 3H), 2.49 (s, 2H), 3.69 (s, 2H), 4.27 (s, 2H), 5.25 (d, 1H, J = 17.86 Hz), 5.48 (d, 1H, J = 11.54 Hz), 6.77 (dd, 1H, J = 11.21 Hz, J = 17.62 Hz), 7.45 (s, 1H); MS (CI) 368 (M + H).

[(7-Bromo-6-ethyl-2,3-dioxo-1,2,3,4-tetrahydroquinox-alin-5-ylmethyl)methylamino]acetic Acid (6). A solution of **2** (0.475 g, 1.27 mmol) in EtOH (75 mL) was hydrogenated (H_2, 50 psi) in the presence of RaNi (0.2 g). The reaction mixture was filtered, and the filtrate was evaporated to give 0.450 g of a white solid, [(2,3-diamino-5-bromo-6-ethylbenzyl)-methylamino]acetic acid ethyl ester (**5**), which was used without further purification. To a stirred solution of **5** (0.450 g) in 5 N HCl (5 mL) was added oxalic acid dihydrate (0.327 g, 2.6 mmol). The reaction mixture was heated to 90 °C for 16 h, after which additional oxalic acid dihydrate (0.163 g, 1.3 mmol) was added. On cooling, an off-white solid separated, which was filtered, washed with water and methanol, and dried to give 0.174 g (34%) of **6**: mp >245−248 °C; ^{1}H NMR (CD_3OD) δ 1.15 (t, 3H, J = 7.5 Hz), 2.84 (s, 3H), 3.11 (q, 2H), 4.32 (s, 2H), 4.71 (s, 2H), 7.56 (s, 1H); MS (CI) 326 (M − CO_2H + 1); HRMS calcd for $C_{14}H_{16}BrN_3O_4$ $(M + 1)^+$ = 370.0402, found 370.0385.

[(2,3-Diamino-6-ethylbenzyl)methylamino]acetic Acid Ethyl Ester (7). A solution of **2** (0.5 g, 1.34 mmol) and KOAc (0.13 g, 1.34 mmol) in EtOH was hydrogenated (H_2, 50 psi) over Pd/C (20%, 0.1 g) in a Parr apparatus. The reaction mixture was filtered and the filtrate evaporated to give 0.42 g of a semisolid, which was used without further purification.

[(6-Ethyl-2,3-dioxo-1,2,3,4-tetrahydroquinoxalin-5-yl-methyl)methylamino]acetic Acid (8). A solution of **7** (0.42 g) and oxalic acid dihydrate (0.327 g, 2.6 mmol) in aqueous 2 N HCl (20 mL) was heated to 90 °C. The reaction mixture was stirred for 16 h and cooled. A buff precipitate separated, which was filtered and air-dried to yield 0.368 g (84%) of **8**, which was used without further purification: ^{1}H NMR (DMSO-d_6) δ 1.09 (t, 3H, J = 7.5 Hz), 2.17 (s, 3H), 2.66 (q, 2H, J = 7.4 Hz, J = 11.1 Hz), 3.39 (s, 2H), 3.76 (s, 2H), 6.96 (d, 1H, J = 8.2 Hz), 7.04 (d, 1H, 8.2 Hz), 11.90 (s, 1H), 11.98 (bs, 1H); MS (CI) m/z 292 (M + 1).

[(6-Ethyl-7-nitro-2,3-dioxo-1,2,3,4-tetrahydroquinoxa-lin-5-ylmethyl)methylamino]acetic Acid Ammonium Salt (9). A dark solution of **8** (0.368 g) in concentrated H_2SO_4 (5 mL) was cooled to 5 °C, and KNO_3 (0.5 g, 5 mmol) was added under stirring. The reaction mixture was allowed to warm to room temperature and quenched with ice after 12 h. The pale-brown residue was filtered, and the mother liquor was made basic by bubbling NH_3. A pale-green precipitate of the ammonium salt of **9** was obtained, which was filtered and air-dried to yield 0.100 g (25.4%): mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 1.12 (t, 3H, J = 7.3 Hz), 2.01 (s, 3H), 2.8 (q, 2H), 2.92 (s, 2H), 3.70 (s, 2H), 7.61 (s, 1H); MS m/z 337 (M + H).

[(6-Ethyl-2,3-dioxo-1,2,3,4-tetrahydroquinoxalin-5-yl-methyl)methylamino]acetic Acid Ethyl Ester (10). A solution of **7** (0.57 g, 1.9 mmol) and dimethyl oxalate (0.472 g, 4 mmol) in EtOH (5 mL) was heated to reflux for 16 h. The solvent was partially evaporated, and the reaction mixture was cooled to give 0.828 g of a buff precipitate. The product was purified by column chromatography (SiO_2, petroleum ether: EtOAc, 95:5 to 75:25) to give 0.274 g (45%) of **10**: ^{1}H NMR ($CDCl_3$) δ 1.17 (t, 3H, J = 7.8 Hz), 1.33 (t, 3H, J = 7.08 Hz), 2.32 (s, 3H), 2.72 (q, 2H, J = 7.5 Hz, J = 15.1 Hz), 3.37 (s, 2H), 3.86 (s, 2H), 4.31 (q, 2H, J = 7.1 Hz, J = 14.2 Hz), 6.98 (d, 1H, J = 8.3 Hz), 7.14 (d, 1H, J = 8.3 Hz), 10.8 (bs, 1H), 12.3 (bs, 1H); MS m/z 320 (M + H).

[(6-Ethyl-7-nitro-2,3-dioxo-1,2,3,4-tetrahydroquinoxa-lin-5-ylmethyl)methylamino]acetic Acid Ethyl Ester (11). To a stirred solution of **10** (0.175 g, 0.57 mmol) in concentrated H_2SO_4 (2 mL) at 5 °C was added KNO_3 (0.071 g, 0.71 mmol). The reaction mixture was stirred for 16 h and poured over ice. The brown precipitate was filtered, washed with water, and purified by column chromatography (SiO_2, $CHCl_3$:MeOH, 95:5 to 80:20) to give 0.135 g (65%) of **11**: mp 110 °C effervescence, 185 °C dec; ^{1}H NMR ($CDCl_3$) δ 1.22 (t, 3H, J = 7.3 Hz), 1.28 (t, 3H, J = 7.3 Hz), 2.28 (s, 3H), 2.77 (q, 2H, J = 7.3 Hz, J = 14.8 Hz), 3.37 (s, 2H), 3.88 (s, 2H), 4.28 (q, 2H, J = 7.1 Hz, J = 14.4 Hz), 7.69 (s, 1H), 11.37 (bs, 1H), 12.66 (s, 1H); MS m/z 365 (M + H).

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