

Synthesis of Chiral

1-(2'-Amino-2'-carboxyethyl)-1,4-dihydro-6,7-quinoxaline-2,3-diones: α -Amino-3-hydroxy-5-methyl-4-isoxazolepropionate Receptor Agonists and Antagonists

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Recently discovered 6,7-disubstituted quinoxaline-2,3-diones, **1**, have been found to antagonize specific binding and functional responses to both α -amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) and kainic acid. Although a variety of studies have analyzed the activity of quinoxaline-2,3-diones with various substitutions at positions 6 and 7, there is little information regarding the effects of *N*-substitution. A racemic mixture of 1-(2'-amino-2'-carboxyethyl)-1,4-dihydroquinoxaline-2,3-dione (QXAA, **2**, R₁ = R₂ = H) has been synthesized from **1** (R₁ = R₂ = H). This compound inhibited specific [³H]AMPA binding but not [³H]kainate binding. IC₅₀ values for QXAA, AMPA, and DNQX were 0.69, 0.012, and 0.74 μ M, respectively. The *R*- and *S*-enantiomers were prepared by asymmetric synthesis. The *S*-isomer (**2b**) was 160-fold more potent in binding assays than the *R*-isomer (**2d**), with IC₅₀ values of 0.23 and 38 μ M, respectively. Both enantiomers were agonists in a functional assay, with the *S*-isomer having an EC₅₀ value of 3 μ M while that for the *R*-isomer was greater than 1 mM. Methyl substitutions at positions 6 and 7 (**2a** and **2c**) resulted in antagonist compounds characterized by the *S*- and *R*-isomers being nearly equipotent, with IC₅₀ values of 51 and 22 μ M in the binding assay and EC₅₀ values of 290 and 300 μ M in the functional assay. AMPA had an EC₅₀ value of 11 μ M and DNQX an EC₅₀ value of 30 μ M in the functional assay. Analogs of quinoxalinediones with side chains other than an amino acid moiety on the nitrogen did not show good binding activities.

Introduction

Glutamate is the major excitatory neurotransmitter in the mammalian brain. Receptors for glutamate fall into three major subtypes: *N*-methyl-D-aspartate (NMDA) and α -amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA)/kainate receptor subtypes, which are associated with ion channels, and the metabotropic subtype, which is linked to G-proteins and second messenger systems.¹ The amount of glutamate available to receptors is normally highly regulated. However, in some disease states, excessive glutamate becomes available. The resultant overstimulation of receptors may result in excitotoxicity, which may be a factor in neuronal damage associated with Parkinson's disease, Alzheimer's disease, and epilepsy. In addition, activation of glutamate receptors appears to be involved in the mechanism of drug addiction. Thus, antagonists at glutamate receptors have the potential to be effective therapies for a variety of neurological disorders. A variety of antagonists of excitatory amino acid receptors have been studied and reported in the literature.²

Cocaine and amphetamine are among the most popular drugs of abuse.³ Our group has been evaluating AMPA/kainate antagonists as potential adjuncts in the treatment of cocaine and amphetamine addiction. Both drugs are powerful central nervous system (CNS) stimulants, which promote euphoria and psychomotor stimulation.⁴ These effects are thought to be mediated

by enhanced dopamine neurotransmission in the nucleus accumbens, since they are antagonized by 6-hydroxy-dopamine-induced lesions of mesolimbic dopamine neurons and dopamine receptor blocking agents injected into the nucleus accumbens.⁵ We have found that these effects are also antagonized by the injection of AMPA/kainate receptor antagonist, 6,7-dinitro-1,4-dihydroquinoxaline-2,3-dione (DNQX) (**1**, R₁ = R₂ = NO₂), into the nucleus accumbens.⁶ These observations suggest that enhanced glutamate neurotransmission as well as dopamine neurotransmission may mediate the addicting effects of cocaine and amphetamine.

The use of AMPA/kainate antagonists for therapeutic purposes would be limited by potency and oral bioavailability. Consequently, one goal of our study is to develop more potent AMPA/kainate antagonists. A few active AMPA antagonists have recently been reported.⁷ Intrigued by the resemblance of the quinoxalinedione structure to that of β -oxalyl-L-alanine (BOAA), an AMPA receptor agonist and a neurotoxin found in *Lathyrus sativus* (Scheme 1), we have synthesized analogs of quinoxalinediones with an amino acid moiety connected to the nitrogen in the quinoxalinedione ring, **2** (quinoxalinedione amino acids, QXAA). In addition, since the AMPA receptor shows a pronounced stereoselectivity toward the L-enantiomers of agonists,⁸ we have prepared optically pure enantiomers of **2** and have compared their biological activities.

Chemistry

Our initial effort to synthesize optically pure 1-(2'-amino-2'-carboxyethyl)-1,4-dihydroquinoxaline-2,3-di-

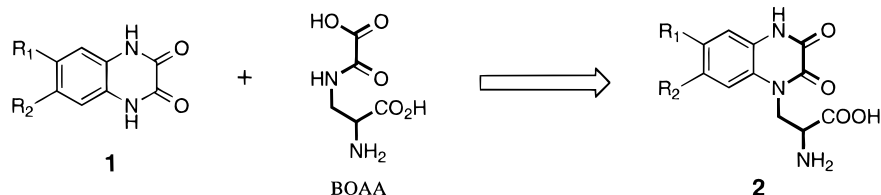
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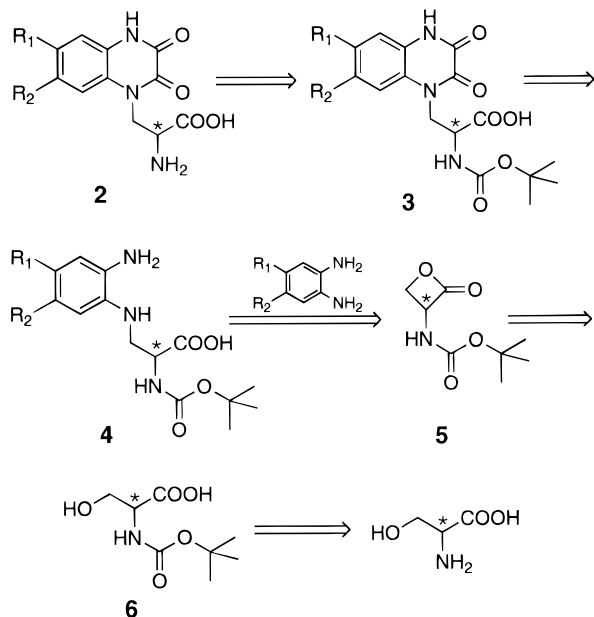
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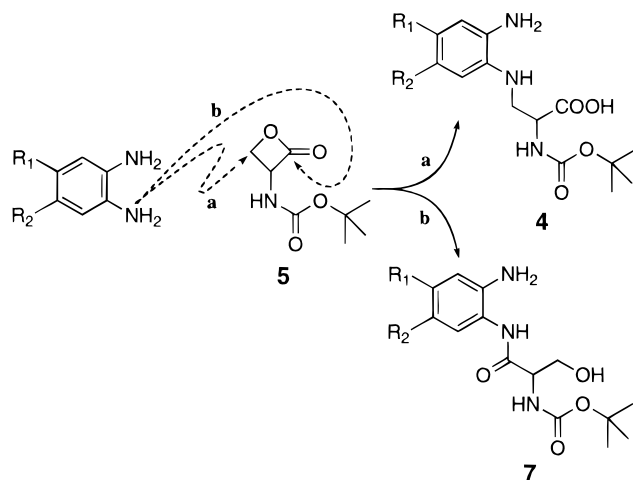
Scheme 1



Scheme 2



Scheme 3

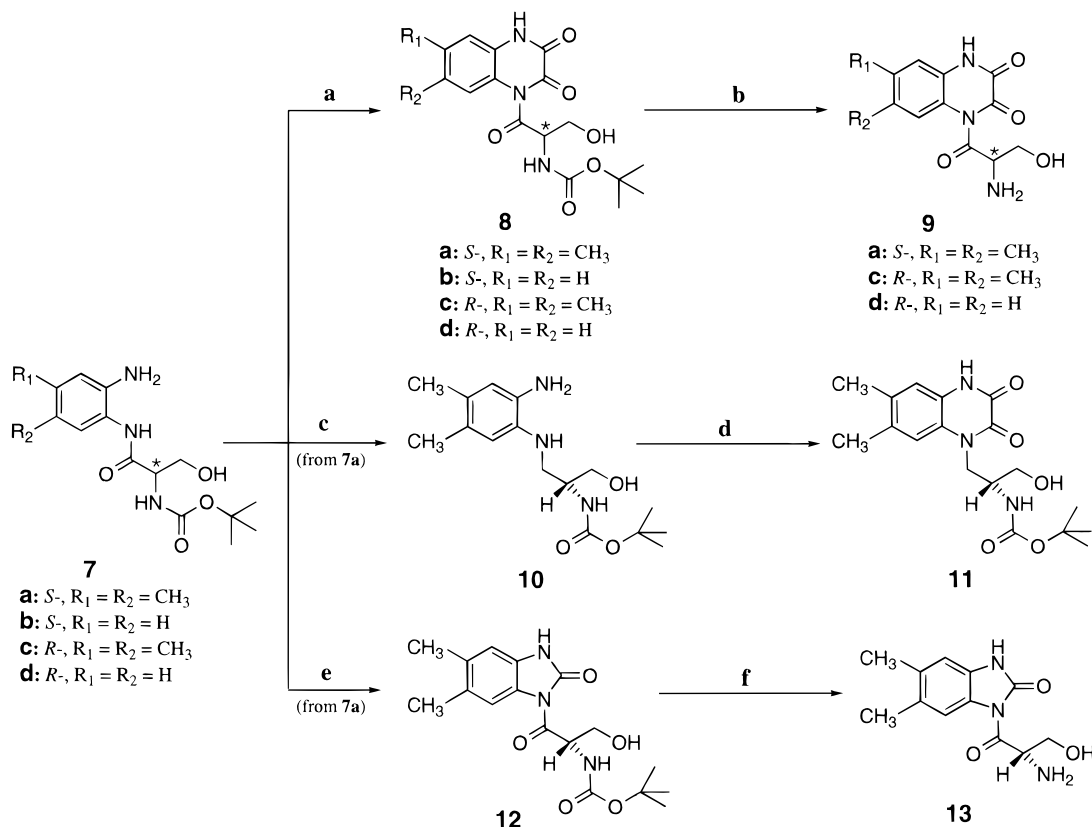


one, QXAA (2), is demonstrated in Scheme 2. *N*-*tert*-Butoxycarbonyl-L-serine β-lactone (5) was prepared from commercially available *N*-*tert*-BOC-L-serine (6) by Mitsunobu cyclization.^{9–12} However, the nucleophilic addition of phenylene-1,2-diamine (R₁ = R₂ = H) or 4,5-dimethylphenylene-1,2-diamine (R₁ = R₂ = CH₃) to the lactone 5 did not give the desired products 4. In fact, compound 7 (Scheme 3) was obtained. This can be explained by the fact that the lactone has two electrophilic sites, the carbonyl carbon (C-1) and the β-carbon (C-3) atoms, and both are capable of reacting with appropriate nucleophiles under certain conditions. Scheme 3 shows the two possible pathways which lead to two different products: (a) nucleophilic attack at the β-carbon (C-3) of the lactone gives β-(arylamino)-*N*-*tert*-

BOC-alanine 4; (b) nucleophilic attack at the carbonyl carbon (C-1) of the lactone with acyl oxygen cleavage generates *N*-aryl-2-(*N*-*tert*-BOC-amino)-3-hydroxypropionamide 7. Although two products, 4 and 7, were expected from these reactions, only the latter was obtained. Encouraged by the literature report,^{10,13} several attempts (e.g., replacing the protection group *N*-*tert*-BOC with *N*-CBZ, varying solvents, adjusting reaction conditions) were made to steer the reaction toward product 4, but all efforts were unsuccessful. Therefore, we considered the possibility of converting the alcohol functional group in 7 to carboxylic acid after the quinoxalinedione ring was introduced. Scheme 4 details the synthesis of *N*-substituted quinoxaline-2,3-diones 8, 9, and their five-membered-ring analogs 12 and 13. However, the oxidation of the hydroxyl group in these compounds with pyridinium dichromate (PDC), (C₅H₅NH⁺)₂Cr₂O₇²⁻, or pyridinium chlorochromate (PCC), C₅H₅NH⁺ ClCrO₃⁻, under various conditions did not give the desired carboxylic acids but gave a mixture of unidentified materials. It appears that these compounds cannot tolerate oxidation, at least under the conditions attempted.

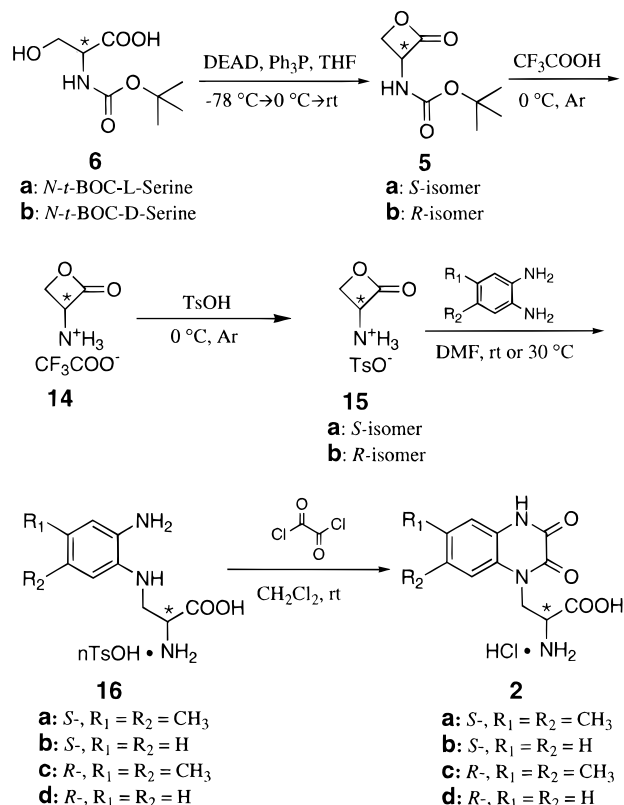
Reduction of 7a with BH₃ in THF at -20 °C followed by the formation of quinoxaline ring furnished 11, an analog of 8. Its oxidation under the same conditions as above also gave a mixture of unidentified compounds. With these results, we turned our attention to the β-lactone ring-opening step. Our goal was to see if we could modify the conditions such that the aromatic amines could open the β-lactone ring by attacking the β-carbon atom (C-3). In oxygen-18-labeling studies of the β-lactone, it was shown that aqueous sodium hydroxide opened the serine β-lactone ring by exclusive attack at the carbonyl carbon (C-1), whereas acidic hydrolysis proceeded primarily by attack of water at the C-3 methylene group.¹⁴ It was also reported that a variety of nucleophiles were able to react at the β-position with 3-amino-2-oxetanone salts, derived from lactone 5 by the treatment with appropriate acids (trifluoroacetic acid, *p*-toluenesulfonic acid).¹⁵ Both literature reports suggested that under acidic conditions nucleophiles opened the β-lactone ring by attack at the C-3 position. Thus, using the same approach, phenylene-1,2-diamine and 4,5-dimethylphenylene-1,2-diamine was treated with the *p*-toluenesulfonic acid salts of (*R*)- and (*S*)-3-amino-2-oxetanone (15) to afford the desired products. In summary, under basic or neutral conditions, nucleophiles tend to attack the carbonyl carbon (C-1) in lactone 5 to yield alcoholic products (e.g. 7 in Scheme 3), while under acidic conditions, lactone 5 is converted to the corresponding salt (e.g. 15), resulting in a C-3 attack by a nucleophile to give a product with an amino acid moiety (e.g. 16 in Scheme 5).

Scheme 5 shows the total syntheses of optically pure QXAA (2). The key intermediate β-lactone 5 was readily

Scheme 4^a

^a (a) (1) 4 equiv of LDA, THF, -78°C , (2) diethyl oxalate, THF, -78°C , (3) H_2O , H^+ (pH = 5); (b) CF_3COOH , room temperature; (c) BH_3 , THF, -20°C ; (d) (the same as a); (e) (1) 4 equiv of LDA, THF, -78°C , (2) diethyl carbonate, THF, -78°C , (3) H_2O , H^+ (pH = 5); (f) CF_3COOH , room temperature.

Scheme 5



prepared from **6** without detectable epimerization. Yields of **5** were improved by extending the reaction time to overnight at -78°C . The rate of addition of **6**

to the triphenylphosphine–azodicarboxylate adduct should be carefully controlled by maintaining the temperature at -78°C in order to minimize the production of *N*-*t*-BOC-aminoethylene, a byproduct generated from the unstable adduct of **6** and Ph_3P via decarboxylation. Treatment of **5** with anhydrous trifluoroacetic acid gave 3-amino-2-oxetanone trifluoroacetate **14**, which was immediately converted to its tosylate salt **15** *in vacuo* by addition of 1 equiv of anhydrous *p*-toluenesulfonic acid. Compound **15** is stable under dry conditions and best stored under argon. Nucleophilic addition of excess amount of 4,5-dimethylphenylene-1,2-diamine to **15a** or **15b** at the β -position (C-3) in dry DMF furnished β -[(2'-amino-4',5'-dimethylphenyl)amino]-L-alanine (**16a**) or β -[(2'-amino-4',5'-dimethylphenyl)amino]-D-alanine (**16c**). The same reaction of phenylene-1,2-diamine with **15a** or **15b** gave β -[(2'-aminophenyl)amino]-L-alanine (**16b**) or β -[(2'-aminophenyl)amino]-L-alanyl-D-alanine (**16d**). These reactions should be conducted under mild conditions (room temperature or 30°C) because **15** is not stable in DMF. High temperatures intensify its decomposition rate. The same phenomenon was observed in DMSO, and the decomposition was even faster. The cause of the decomposition remains unknown. Cyclization of **16** with a solution of oxalyl chloride in methylene chloride readily produced optically pure QXAA (**2**).

In order to confirm the optical purity of the final compounds, enantiomeric isomers **2a/2c** and **2b/2d** were carefully derivatized with 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide (FDAA, Marfey's reagent) under mild conditions¹⁶ and separated and quantitated by reverse-phase HPLC.¹⁷ The nature of the reagent and the

Table 1. Percent Inhibition of Specific [³H]Kainate and [³H]AMPA Binding by 100 μ M Test Compound

| compd | config | substitutions | [³ H]kainate | [³ H]AMPA |
|------------|----------|---|--------------------------|-----------------------|
| 7a | <i>S</i> | R ₁ = R ₂ = CH ₃ | 11 ± 7 | 4 ± 8 |
| 8a | <i>S</i> | R ₁ = R ₂ = CH ₃ | 11 ± 9 | 12 ± 6 |
| 11 | <i>R</i> | | 5 ± 13 | 47 ± 3 |
| 9a | <i>S</i> | R ₁ = R ₂ = CH ₃ | 15 ± 5 | 14 ± 4 |
| 9c | <i>R</i> | R ₁ = R ₂ = CH ₃ | 16 ± 14 | 24 ± 6 |
| 13 | <i>S</i> | | 13 ± 13 | 21 ± 8 |
| 15a | <i>S</i> | | 5 ± 19 | 11 ± 12 |
| 15b | <i>R</i> | | 4 ± 1 | 0 ± 8 |
| 16a | <i>S</i> | R ₁ = R ₂ = CH ₃ | 7 ± 15 | 15 ± 11 |
| 16c | <i>R</i> | R ₁ = R ₂ = CH ₃ | 0 ± 6 | 15 ± 3 |
| 16b | <i>S</i> | R ₁ = R ₂ = H | 14 ± 16 | 70 ± 5 |
| 16d | <i>R</i> | R ₁ = R ₂ = H | 0 ± 3 | 0 ± 8 |
| 2a | <i>S</i> | R ₁ = R ₂ = CH ₃ | 38 ± 1 | 66 ± 3 |
| 2c | <i>R</i> | R ₁ = R ₂ = CH ₃ | 27 ± 1 | 62 ± 6 |
| 2b | <i>S</i> | R ₁ = R ₂ = H | 27 ± 5 | 95 ± 3 |
| 2d | <i>R</i> | R ₁ = R ₂ = H | 5 ± 4 | 77 ± 3 |
| DNQX | | | 81 ± 5 | 99 ± 0.4 |

resultant reaction products with D-diastereomers (i.e. **2c** and **2d**) suggest that strong intramolecular hydrogen bonding causes these derivatives to elute much later than their L-diastereomer counterparts (i.e. **2a** and **2b**). The results were consistent with the theory and demonstrated that **2a** had an optical purity of >99% ee (with retention time $t_R = 2.49$ min) and **2c** >95% ee (with retention time $t_R = 3.65$ min), while **2b** >99% ee ($t_R = 2.14$ min) and **2d** >96% ee ($t_R = 3.21$ min).

Biological Results

The biological activity of various quinoxalinedione analogs was evaluated initially by determining the ability of 100 μ M compound to inhibit the binding of [³H]AMPA and [³H]kainate to brain homogenates. In preliminary studies, we found that the racemic mixture of QXAA, **2** (R₁ = R₂ = H), inhibited [³H]AMPA binding with an IC₅₀ value of 0.69 μ M, intermediate between the value of 0.012 μ M for AMPA and the reported value of 1.3 μ M for BOAA.¹⁸ The parent QX, **1** (R₁ = R₂ = H), was inactive at 100 μ M. We, therefore, studied the effects of the individual stereoisomers as well as several intermediates in their preparation and structural analogs with various substituents on the nitrogen. Compounds (**2a–d**) containing an alanine substitution on the nitrogen were active, and these were more effective in inhibiting [³H]AMPA binding than [³H]kainate binding (Table 1). The alanine-substituted compound lacking the diketone function, **16b**, was also active. Analogs of quinoxalinediones with side chains other than an amino acid moiety on the nitrogen in the quinoxalinedione ring were inactive (<50% competition for binding sites). Thus, the amino acid moiety on the *N*-side chain in this series of compounds must play an important role in binding.

Compounds active at 100 μ M were evaluated at several concentrations to determine the potency. On the basis of their IC₅₀ values for inhibition of [³H]AMPA binding (Figure 1), most of the QXAAs were less potent than DNQX. Only the *S*-enantiomer, **2b**, had a potency that was in the range of that of DNQX and, in fact, was slightly more potent. It is interesting that there is a marked stereoisomeric effect on potency for the compounds with H in the 6 and 7 positions, while there is little difference in potency between the isomers with the 6,7-dimethyl substitutions.

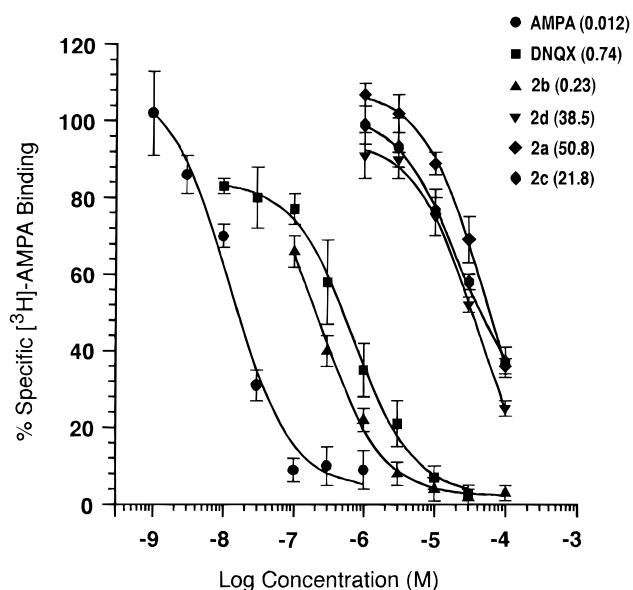


Figure 1. Effects of AMPA, DNQX, and the selected compounds on [³H]AMPA binding. The IC₅₀ value is given next to each compound. 95% Confidence limits of the IC₅₀ values for each compound were AMPA 0.0057–0.028 μ M, DNQX 0.57–0.96 μ M, **2b** 0.15–0.38 μ M, **2d** 18–81 μ M, **2a** 28–93 μ M, and **2c** 12–40 μ M.

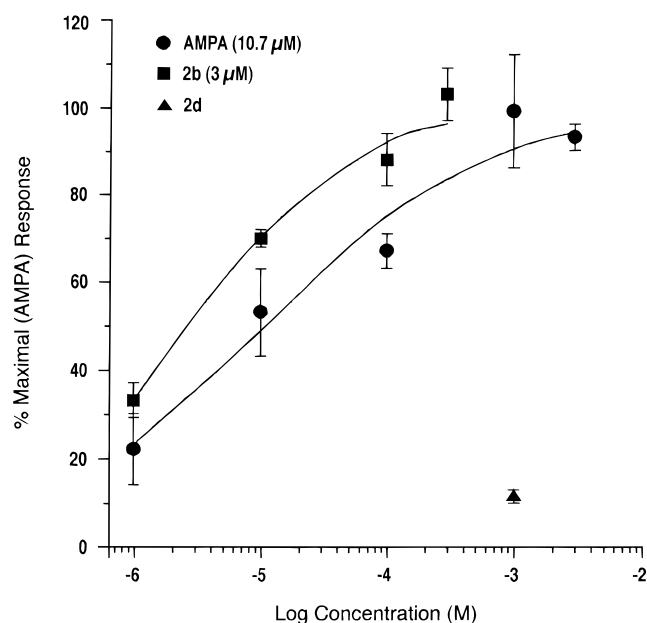


Figure 2. Effects of the selected compounds on [³H]norepinephrine release from hippocampal slices. 95% Confidence limits of the EC₅₀ values for each compound were AMPA 3.0–30 μ M and **2b** 0.65–8.0 μ M.

For determination of whether compounds showing high potency in the binding assays are functional agonists or antagonists, an assay was used that measures [³H]norepinephrine release from hippocampal slices exposed to AMPA/kainate receptor agonists. Compound **2b** is a potent agonist, with an EC₅₀ value of 3 μ M (Figure 2). This was equipotent with kainate and about 3-fold more potent than AMPA in this assay system. These responses were blocked by DNQX (data not shown). The *R*-stereoisomer, **2d**, was a weak agonist, producing less than 50% stimulated release at a concentration of 1 mM (Figure 2). However, compounds **2a** and **2c** were effective antagonists (Figure 3). The *R*- and the *S*-stereoisomers were nearly equi-

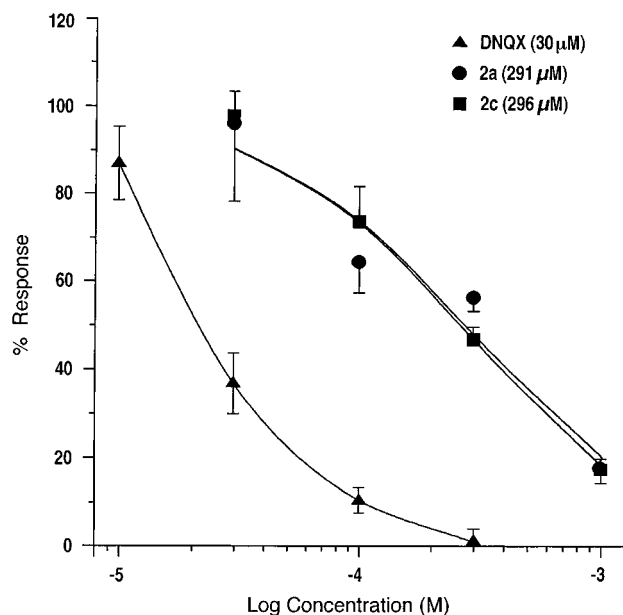


Figure 3. Inhibition by selected compounds of AMPA-stimulated release of [³H]norepinephrine from hippocampal slices. 95% Confidence limits of the EC₅₀ values for each compound were **2a** 150–1000 μM, **2c** 200–400 μM, and DNQX 9–60 μM.

potent as antagonists of AMPA-induced [³H]norepinephrine release, with EC₅₀ values of 300 (**2c**, *R*) and 290 μM (**2a**, *S*), respectively. It appears that the type of substitution at the 6- and 7-positions on the dihydroquinoxaline-2,3-dione ring system plays a major role in determining the functional activity. We plan to examine this more in the future.

In addition to their antagonist activity at AMPA receptors, the quinoxalinedione compounds, as a class, have been shown to interact with the glycine site of the NMDA receptor, producing weak to moderate antagonism of NMDA-induced responses.¹⁹ The aforementioned literature points out that many of the standard DNQX-like compounds retain at least 5–6-fold selectivity for non-NMDA receptors and that substituting the molecule at the 5-position enhances glycine site activity. Since the compounds used in this study are exclusively substituted at the 6,7-positions of the benzene ring, we would not predict significant glycine site activity. Nevertheless, we cannot conclude that the quinoxalinedione derivatives synthesized in this study are devoid of NMDA receptor interactions, and we plan to examine this issue more in the future.

Discussion

Incorporating the carbon backbone of the neurotoxin BOAA into the molecule quinoxaline-2,3-dione led to the production of several analogs with a variety of *N*-linked substitutions. Given the affinity of BOAA for the AMPA receptor we hypothesized that hybridizing these two molecules would lead to antagonists with greater AMPA receptor affinity. Indeed, compound **2** (racemic, R₁ = R₂ = H) did exhibit higher affinity for the receptor (0.69 μM) than the >204 μM reported for the unsubstituted quinoxaline-2,3-dione.¹⁹ To our surprise, however, we found that the unsubstituted compound QXAA (**2b** and **2d**, R₁ = R₂ = H) exhibited potent agonist activity, with the *S*-isomer greater than 300-fold more potent than the *R*-isomer. In contrast, dimethyl substitutions at the 6-

and 7-positions resulted in compounds (**2a** and **2c**) that were modest antagonists, with no stereoselectivity. These findings suggest that there may be very rigid binding requirements, possibly two distinct sites, between agonists and antagonists of the AMPA receptor. A certain degree of steric tolerance has been proposed for the AMPA receptor binding pocket,²⁰ and it may be that the lack of 6,7-substitutions allows the benzene ring portion to function as a bioisostere of the position 5 methyl on AMPA. Thus, the primary interaction with the receptor would reside in the diketo and amino acid regions of the molecule. The fact that *S*-isomer stereoselectivity is observed with compound **2b** is consistent with the active *S*-isomers of glutamate and AMPA.

The observation that compound **16b** showed moderate AMPA receptor affinity bears some consideration. We would expect, given the aforementioned concepts, that this compound would behave as an agonist, while the methyl-substituted compounds **16a** and **16c** would function as antagonists. However, the affinity of **16a** and **16c** was quite low, indicating that the diketo portion of the quinoxalinedione is essential for binding to the "antagonist site". This could also explain, at least in partial, the lack of significant receptor interaction shown by compound **13**.

Conclusions

In summary, we found that linking an amino acid residue to the position 1 nitrogen of quinoxaline-2,3-dione resulted in a compound (**2**) with potent AMPA receptor affinity. Substitutions at positions 6 (R₁) and 7 (R₂) of the benzene ring dictated functional activity, such that no substitution (i.e. H) resulted in potent, stereoselective agonist activity, while dimethyl substitutions resulted in modest, nonstereoselective antagonist activity. Modification of the amino acid residue significantly diminished activity of all analogs. These data suggest that AMPA receptor agonists and antagonists may bind to distinct regions of the receptor. This information should lead to a more targeted approach in the design of AMPA receptor antagonists.

Experimental Section

General. The solvents employed in these experiments were purchased and were not further purified unless otherwise specified. Some solvents, if necessary, were dried and distilled over appropriate drying agents, e.g. CH₂Cl₂, CH₃CN, DMF, and diisopropylamine (DIPA) were dried and distilled over calcium hydride (CaH₂) and stored over 4 Å molecular sieves; tetrahydrofuran (THF) was distilled over Na/benzophenone and used immediately; CF₃COOH was distilled over P₂O₅ and stored under Ar. Melting points were determined in capillary tubes with a Thomas-Hoover Unimelt melting point apparatus and are uncorrected. Optical rotations were obtained on Rudolph Autopol III automatic polarimeter in a 1 dm cell in CH₃OH. ¹H and ¹³C NMR spectra were obtained on a Bruker-300 spectrometer. ¹H chemical shifts are reported in ppm relative to CDCl₃ (δ 7.24), CD₂Cl₂ (δ 5.32), CD₃COCD₃ (δ 2.04), DMF-*d*₆ (δ 2.91), and DMSO-*d*₆ (δ 2.49). ¹³C chemical shifts are reported in ppm relative to CDCl₃ (δ 77.00), CD₂Cl₂ (δ 53.80), CD₃COCD₃ (δ 29.8), DMF-*d*₆ (δ 35.2), and DMSO-*d*₆ (δ 39.50). The abbreviations used are as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. NMR data of residual components (such as solvents) were not reported. Baker precoated silica gel Si 500F UV254 plates (500 mm) were employed for analytical thin-layer chromatography (TLC) and Merck silica gel (purchased from Aldrich, grade 9385, 230–400 mesh, 60 Å) for flash column chroma-

tography. Yields are reported after purification. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA.

***N*-(*tert*-Butoxycarbonyl)-L-serine β -Lactone (5a).** A 1000 mL three-neck flask was equipped with a mechanic stirrer, a low-temperature thermometer, and a 300 mL dropping funnel. All glassware were flame-dried and cooled to room temperature under Ar before use. To the flask were added triphenylphosphine (Ph₃P) (26.23 g, 100 mmol, dried over P₂O₅ under vacuum for 72 h) and freshly dried THF (500 mL). The solution was stirred and cooled to -78°C with a dry ice-acetone bath under Ar. With vigorously stirring, freshly distilled diethyl azodicarboxylate (DEAD) (16.5 mL, 105 mmol) was added with a syringe over a period of 30 min. After the addition was complete, the mixture was continually stirred until a milky paste was formed (ca. 30 min). A solution of *N*-(*tert*-butoxycarbonyl)-L-serine (6a) (20.52 g, 100 mmol, dried over P₂O₅ under vacuum for 48 h) in dry THF (200 mL) was added dropwise to the reaction mixture over a period of ca. 30 min. The mixture was then stirred at -78°C overnight under Ar and allowed to warm to 0°C (placed the flask in an ice bath when the temperature reached -10°C). After 30 min, the ice bath was replaced with a H₂O bath, and the reaction mixture was stirred for 2 h and concentrated on a rotary evaporator to a pale yellow oil at 30°C . The oil was treated with 25% EtOAc/hexanes (200 mL), the resulting white solid was removed by filtration and washed with 25% EtOAc/hexanes (2 \times 70 mL), the combined filtrate was concentrated, and the residual oil was subjected to flash column chromatography on silica gel (400 g; column size: 70 \times 170 mm) with 25% (500 mL) and 30% (1500 mL) EtOAc/hexanes successively. Fractions 10–40 (ca. 1200 mL) gave 5a as a white solid: TLC $R_f = 0.27$ (30% EtOAc/hexanes); yield 10.85 g (58%); mp $119\text{--}121^{\circ}\text{C}$ dec (lit.¹⁰ mp $119.5\text{--}120.5^{\circ}\text{C}$ dec); ¹H NMR (CD₂Cl₂) δ 1.45 (s, 9H), 4.35–4.45 (dd, $J_1 = 6.5$ Hz, $J_2 = 4.8$ Hz, 2H), 4.93–5.08 (dd, $J = 5.1$ Hz, 1H), 5.33–5.48 (s, br, 1H); ¹³C NMR (CD₂Cl₂) δ 28.31, 60.01, 66.63, 81.50, 155.01, 169.94. Anal. (C₈H₁₃NO₄) C, H, N.

***N*-(*tert*-Butoxycarbonyl)-D-serine β -Lactone (5b).** Under Ar, freshly distilled DEAD (7.9 mL, 50.01 mmol) was slowly added with a syringe to a solution of Ph₃P (12.51 g, 47.71 mmol, dried over P₂O₅ under vacuum for 72 h) in THF (300 mL) over a period of 20 min at -78°C with stirring. The mixture was continuously stirred for 10 min more until a milky paste was generated. *N*-(*tert*-Butoxycarbonyl)-D-serine (6b) (9.79 g, 47.71 mmol, dried over P₂O₅ under vacuum for 48 h) in 100 mL of THF was added dropwise in a period of 20 min, and the resulting mixture was stirred at -78°C overnight, allowed to warm to 0°C and room temperature slowly, and stirred at room temperature for 2 h. Removal of the solvent on a rotary evaporator at 30°C gave a pale oil. The oil was treated with 25% EtOAc/hexanes (150 mL), and the solid produced was filtered and washed with 25% EtOAc/hexanes (50 mL). The combined filtrates were concentrated to an oil, and the oil was applied to a flash silica gel column chromatography (300 g of silica gel, column size: 70 \times 150 mm), eluting with 25% (500 mL) and 35% (1000 mL) EtOAc/hexanes successively. Fractions 4–33 (ca. 970 mL) gave 5b as a white solid: yield 4.50 g (50%); mp $119\text{--}121^{\circ}\text{C}$; ¹H NMR (CD₂Cl₂) δ 1.44 (s, 9H), 4.35–4.45 (dd, $J_1 = 6.5$ Hz, $J_2 = 4.7$ Hz, 2H), 4.93–5.08 (dd, $J = 5.8$ Hz, 1H), 5.40–5.55 (s, br, 1H); ¹³C NMR (CD₂Cl₂) δ 28.26, 59.94, 66.63, 81.47, 155.01, 169.96. Anal. (C₈H₁₃NO₄) C, H, N.

(*S*)-*N*-(2'-Amino-4',5'-dimethylphenyl)-2-(*N*-(*tert*-butoxycarbonyl)amino)-3-hydroxypropionamide (7a) and Its *R*-Enantiomer 7c. To a solution of 4,5-dimethylphenylene-1,2-diamine (2.18 g, 16.05 mmol) in THF (20 mL) was added dropwise 5a (2.00 g, 10.7 mmol) in THF (30 mL) at 30°C with stirring. After the addition was complete, the solution was stirred at 30°C overnight. Removal of the solvent in vacuum at 30°C gave a pale yellow oil which was applied to a silica gel flash column chromatography, eluting with CH₂-Cl₂ (500 mL) and 1–3% MeOH/CH₂Cl₂ successively. Fractions 10–30 (ca. 500 mL) gave 7a as an off-white solid: yield 2.19 g (63%); mp $136\text{--}138^{\circ}\text{C}$; ¹H NMR (DMSO-*d*₆) δ 1.38 (s, 9H),

2.03 (s, 3H), 2.06 (s, 3H), 3.55–3.68 (m, 2H), 4.02–4.15 (dd, 1H), 4.48–4.58 (s, br, 2H), 4.90–5.00 (t, 1H, OH), 6.48 (s, 1H), 6.81 (s, 1H), 9.01 (s, 1H); ¹³C NMR (DMSO-*d*₆) δ 18.32, 19.09, 28.14, 56.86, 61.92, 78.23, 116.91, 120.38, 123.16, 127.02, 133.76, 140.28, 155.24, 169.22. Anal. (C₁₆H₂₅N₃O₄·H₂O) C, H, N. The *R*-isomer, 7c, was prepared similarly from 5b with 61% yield: off-white solid; mp $139\text{--}141^{\circ}\text{C}$.

(*R*)-*N*-(2'-Aminophenyl)-2-(*N*-(*tert*-butoxycarbonyl)amino)-3-hydroxypropionamide (7d) and Its *S*-Enantiomer 7b. A mixture of well-dried phenylene-1,2-diamine (3.78 g, 35.0 mmol) in freshly distilled THF (15 mL) was warmed and stirred until a clear solution formed. Under Ar, the brown solution was allowed to cool to room temperature prior to the addition of 5b (1.87 g, 10.0 mmol). After the addition was complete, the solution was stirred at room temperature overnight and at ca. 30°C for 5 h when solid precipitated out. CH₂Cl₂ (ca. 20 mL) was then added, and after the mixture was allowed to cool at room temperature the solid was collected by filtration. After being washed with ether and dried in the air, 0.68 g of white solid was obtained. The combined mother liquid was evaporated over a rotary evaporator at 30°C , and the residual solid was applied to a silica gel column. The column was eluted with 2% MeOH/CH₂Cl₂ (800 mL), and fractions 3–5 (ca. 200 mL) were collected and concentrated to a solid. Treatment of this material with CH₂Cl₂ and ether gave 0.51 g of white solid. Thus, a total of 1.19 g of white solid identified as 7d was obtained: yield 40%; mp $156\text{--}158^{\circ}\text{C}$; ¹H NMR (CD₃COCD₃) δ 1.43 (s, 9H), 3.78–3.85 (m, 1H), 3.91–3.98 (m, 1H), 4.27–4.33 (dd, 1H; t, 1H, OH), 4.54 (s, br, 2H), 6.14 (s, br, 1H), 6.60 (ddd, $J_{5,6} = J_{5,4} = 7.7$ Hz, $J_{5,3} = 1.4$ Hz, 1H), 6.77 (dd, $J_{3,4} = 8.0$ Hz, $J_{3,5} = 1.2$ Hz, 1H), 6.94 (ddd, $J_{4,3} = J_{4,5} = 8.0$ Hz, $J_{4,6} = 1.4$ Hz, 1H), 7.21 (m, 1H), 8.61 (s, 1H); ¹³C NMR (CD₃COCD₃) δ 28.54, 57.72, 63.40, 79.70, 117.03, 117.83, 124.41, 126.78, 127.38, 143.35, 156.53, 170.20. Anal. (C₁₄H₂₁N₃O₄) C, H, N. The *S*-isomer, 7b, was prepared similarly from 5a with 35% yield: white solid; mp $157\text{--}159^{\circ}\text{C}$. Anal. (C₁₄H₂₁N₃O₄) C, H, N.

(*S*)-1-(2'-((*tert*-Butoxycarbonyl)amino)-3'-hydroxypropionyl)-1,4-dihydro-6,7-dimethylquinoxaline-2,3-dione (8a). Under Ar, BuLi (2.5 N in hexanes, 2.7 mL, 6.75 mmol) was slowly added to a solution of diisopropylamine (0.84 mL, 6.0 mmol) in THF (7 mL) at -78°C . After the addition was complete, the solution was allowed to warm to 0°C and stirred for 30 min, and cooled to -78°C again prior to the addition of 7a (0.48 g, 1.41 mmol) in THF (6 mL). After being stirred at 0°C for 30 min, the solution was cooled to -78°C and diethyl oxalate (0.25 mL, 1.8 mmol) in 2 mL of THF was added. The dark orange solution faded to light orange after 30 min. The solution was then allowed to warm to room temperature, stirred overnight, and poured to a mixture of crushed ice and H₂O, 5% HCl was added until pH ~ 5 , and the mixture was extracted with CH₂Cl₂ (3 \times 50 mL). The combined extracts were dried over anhydrous MgSO₄ and concentrated. The residual solid was recrystallized from ether-hexanes, filtered, washed with hexanes, and dried to furnish 224 mg (42%) of 8a as an off-white solid: mp $239\text{--}241^{\circ}\text{C}$ dec; ¹H NMR (DMSO-*d*₆) δ 1.37 (s, 9H), 2.21 (s, 3H), 2.30 (s, 3H), 3.50–3.65 (m, 2H), 4.10–4.16 (m, 1H), 4.92 (t, 1H, -OH), 6.74–6.80 (d, $J = 7.7$ Hz, 1H), 7.16 (s, 1H), 7.52 (s, 1H), 9.99 (s, 1H); ¹³C NMR (DMSO-*d*₆) δ 19.02, 19.11, 28.16, 57.10, 61.70, 78.34, 124.99, 125.96, 127.34, 127.51, 127.59, 133.65, 133.95, 155.22, 157.80, 170.03; $[\alpha]_D^{20} = -43.3^{\circ}$ (c 1.40, CH₃OH). Anal. (C₁₈H₂₃N₃O₆) C, H, N. The *R*-isomer, 8c, was prepared similarly from 7c with 33% yield: pale yellow solid; mp $220\text{--}230^{\circ}\text{C}$ dec.

(*S*)-1-(2'-((*tert*-Butoxycarbonyl)amino)-3'-hydroxypropionyl)-1,4-dihydroquinoxaline-2,3-dione (8b). Following the procedure described for 8a, 8b was prepared from 7b (0.44 g, 1.5 mmol), BuLi (2.5 N in hexanes, 2.7 mL, 6.75 mmol), and diethyl oxalate (0.22 mL, 1.6 mmol): white solid; yield 250 mg (48%); mp $206\text{--}208^{\circ}\text{C}$ dec; ¹H NMR (DMSO-*d*₆) δ 1.36 (s, 9H), 3.31–3.65 (m, 2H), 4.10–4.16 (m, 1H), 4.94 (t, br, 1H, OH), 6.78–6.81 (d, $J = 7.5$ Hz, 1H), 7.05–7.71 (m, 4H), 9.81 (s, 1H); ¹³C NMR (DMSO-*d*₆) δ 28.09, 57.23, 61.72, 78.30, 122.94, 124.53, 125.08, 125.44, 125.89, 129.64, 130.35, 155.22, 158.03, 170.13. The *R*-isomer, 8d, was prepared similarly from 7d with 51% yield: white solid; mp $203\text{--}205^{\circ}\text{C}$ dec.

(S)-1-(2'-Amino-3'-hydroxypropionyl)-1,4-dihydro-6,7-dimethylquinoxaline-2,3-dione (9a). A mixture of **8a** (200 mg, 0.53 mmol) and CF₃COOH (3 mL) was stirred overnight at room temperature. The resulting suspension was filtered, and the solid was washed with H₂O and ether and dried over P₂O₅ under vacuum to furnish 130 mg (87%) of **9a** as a white solid: mp 254–256 °C dec; ¹H NMR (DMSO-*d*₆) δ 2.21 (s, 3H), 2.22 (s, 3H), 3.75–3.90 (m, 2H), 3.95–4.02 (m, 1H), 5.60 (t, 1H, OH), 7.23 (s, 1H), 7.51 (s, 1H), 8.15–8.35 (s, br, 2H), 9.93 (s, 1H); ¹³C NMR (DMSO-*d*₆) δ 19.10, 19.19, 54.84, 60.46, 125.26, 126.02, 127.02, 127.41, 134.31, 158.02, 166.31. Anal. (C₁₃H₁₅N₃O₄) C, H. The *R*-enantiomer, **9c**, was prepared similarly from **8c** with 63% yield: white solid; mp 230 °C dec. **9d** was also prepared from **8d** with 65% yield: off-white solid; mp 195–200 °C dec.

(R)-2-((tert-Butoxycarbonyl)amino)-3-((2'-amino-4',5'-dimethylphenyl)amino)propan-1-ol (10) and (R)-1-(2'-((tert-Butoxycarbonyl)amino)-3'-hydroxypropionyl)-1,4-dihydro-6,7-dimethylquinoxaline-2,3-dione (11). To a suspension of **7a** (1.22 g, 3.77 mmol) in THF (12 mL) was added BH₃ (1 M in THF, 18.9 mL, 18.85 mmol) at 0 °C with a syringe. The mixture was stirred at 0 °C for 3 h and at room temperature overnight. CH₃OH (15 mL) was then added, and the mixture was stirred at room temperature overnight. Concentration of the resulting solution under vacuum gave a pale oil. The oil was treated with CH₃OH (3 × 30 mL) with evaporation to dryness after each addition to remove boric acid as trimethyl borate [B(OCH₃)₃]. The solid thus obtained was recrystallized from CH₂Cl₂-ether-hexanes to give 1.07 g (91%) of **10** as a white solid: mp 102–104 °C; ¹H NMR (CDCl₃) δ 1.43 (s, 9H), 2.10 (s, 3H), 2.14 (s, 3H), 3.15–3.35 (AB, 2H), 3.77–3.80 (m, 2H), 3.82–3.88 (m, 1H), 5.02–5.11 (s, br, 1H, OH), 6.48 (s, 1H), 6.51 (s, 1H). Compound **11** was prepared according to the procedure described for **8a** using **10** (0.53 g, 1.71 mmol), LDA (prepared from diisopropylamine and BuLi), and diethyl oxalate (0.28 mL, 2.05 mmol) and was isolated as a pale yellow solid: yield 0.35 g (56%); mp 241–243 °C dec; ¹H NMR (DMSO-*d*₆) δ 1.18 (s, 9H), 2.18 (s, 3H), 2.25 (s, 3H), 3.38–3.48 (m, 2H), 3.80–3.90 (m, 1H), 4.05–4.15 (m, 2H), 4.88 (t, 1H, OH), 6.54–6.57 (d, *J* = 8.9 Hz, 1H), 6.90 (s, 1H), 7.34 (s, 1H), 11.87 (s, 1H); ¹³C NMR (DMSO-*d*₆) δ 18.69, 19.23, 27.85, 44.00, 50.54, 61.51, 77.58, 115.92, 116.12, 123.29, 124.54, 131.17, 131.40, 153.39, 155.30, 155.55. Anal. (C₁₈H₂₅N₃O₅) C, H.

(S)-1-(2'-((tert-Butoxycarbonyl)amino)-3'-hydroxypropionyl)-1,3-dihydro-5,6-dimethylbenzimidazol-2-one (12) and (S)-1-(2'-Amino-3'-hydroxypropionyl)-1,3-dihydro-5,6-dimethylbenzimidazol-2-one (13). Treatment of **7a** (1.00 g, 3.00 mmol) with excess LDA [prepared from BuLi (2.5 N in hexanes, 5.4 mL, 13.5 mmol) and diisopropylamine (1.7 mL, 12.00 mmol)] and diethyl carbonate (0.44 mL, 3.6 mmol) (detailed procedure refers to the preparation of **8a**) gave **12** as a pale yellow solid: yield 0.65 g (62%); mp 135–137 °C; ¹H NMR (CDCl₃) δ 1.44 (s, 9H), 2.07 (s, 3H), 2.09 (s, 3H), 3.60–3.65 (m, 2H), 3.95–4.05 (m, 1H), 4.20–4.30 (t, br, 1H, OH), 5.80–5.88 (d, br, 1H), 6.50 (s, 1H), 6.85 (s, 1H), 8.29 (s, 1H). A mixture of **12** (0.60 g, 1.72 mmol), acetone (2 mL), and CF₃COOH (3 mL) was stirred overnight at room temperature. H₂O (20 mL) was introduced, and the mixture was washed with ether (3 × 50 mL). The aqueous layer was concentrated to dryness under vacuum. Recrystallization of the residual solid from CH₃OH-ether-hexanes gave a pale yellow solid identified as **13**: yield 0.36 g (84%); mp 180–182 °C; ¹H NMR (DMSO-*d*₆) δ 2.04 (s, 3H), 2.07 (s, 3H), 3.80–3.90 (m, 2H), 3.92–4.01 (m, 1H), 4.45–4.85 (s, br, 2H), 5.63 (t, br, 1H, OH), 6.52 (s, 1H), 6.84 (s, 1H), 9.54 (s, 1H); ¹³C NMR (DMSO-*d*₆) δ 18.37, 19.13, 54.60, 60.37, 117.03, 119.30, 123.31, 126.94, 134.44, 140.35, 157.88, 165.72. Anal. (C₁₂H₁₅N₃O₃) C, H.

(S)-3-Amino-2-oxetanone *p*-Toluenesulfonic Acid Salt (15a). A solution of TsOH²¹ (4.63 g, 26.9 mmol) in dry CF₃COOH (60 mL) was stirred under Ar and cooled to 0–5 °C in an ice bath. **5a** (4.79 g, 25.6 mmol, dried over P₂O₅ under vacuum overnight) was added in one portion. After the addition was complete, the resulting solution was stirred at 0–5 °C for 15 min and concentrated on a rotary evaporator at 30 °C. The solid, thus obtained, was treated with anhydrous

ether, separated by filtration, washed with ether, and dried over P₂O₅ under vacuum, and 6.1 g (92%) of **15a** was obtained as a white solid: mp 135 °C (darkening), 170 °C (decomposed rapidly) (lit.¹⁵ mp 135–173 °C dec); ¹H NMR (DMF-*d*₆) δ 2.32 (s, 3H), 4.67–4.75 (m, 2H), 5.52–5.55 (dd, *J*₁ = 4.7 Hz, *J*₂ = 6.2 Hz, 1H), 7.15–7.18 (d, *J* = 7.8 Hz, 2H), 7.65–7.68 (d, *J* = 8.0 Hz, 2H), 9.30–9.90 (s, br, 3H); ¹³C NMR (DMF-*d*₆) δ 21.00, 57.85, 65.06, 126.42, 128.87, 139.08, 146.10, 166.22. Anal. (C₁₀H₁₃NO₅S) C, H, N, S.

(R)-3-Amino-2-oxetanone *p*-Toluenesulfonic Acid Salt (15b). Under Ar, **5b** (4.00 g, 21.37 mmol, dried over P₂O₅ under vacuum overnight) was added in one portion to a solution of TsOH (3.86 g, 22.44 mmol) in dry CF₃COOH (40 mL) at 0–5 °C with stirring. The resulting solution was stirred at 0–5 °C for 15 min and concentrated on a rotary evaporator at 30 °C to dryness. The solid was treated with anhydrous ether, separated by filtration, washed with ether, and dried over P₂O₅ under vacuum; 4.24 g (77%) of **15b** was obtained as a white solid: mp 135 °C (darkening), 170 °C (decomposed rapidly); ¹H NMR (DMF-*d*₆) δ 2.32 (s, 3H), 4.67–4.75 (m, 2H), 5.52–5.55 (dd, *J*₁ = 4.7 Hz, *J*₂ = 6.4 Hz, 1H), 7.15–7.18 (d, *J* = 7.8 Hz, 2H), 7.65–7.68 (d, *J* = 8.1 Hz, 2H), 9.20–9.90 (s, br, 3H); ¹³C NMR (DMF-*d*₆) δ 21.00, 57.85, 65.06, 126.42, 128.88, 139.12, 146.05, 166.23. Anal. (C₁₀H₁₃NO₅S) C, H, N, S.

β-((2'-Amino-4',5'-dimethylphenyl)amino)-L-alanine (16a). A solution of 4,5-dimethylphenylene-1,2-diamine (1.43 g, 10.5 mmol, dried over P₂O₅ under vacuum overnight) in dry DMF (10 mL) was stirred under Ar and cooled to 0 °C in an ice bath prior to the addition of **15a** (0.78 g, 3 mmol). The resulting solution was stirred at 0 °C for 2 h and at 27 °C (H₂O bath) for 2 days when solid material precipitated out. The mixture was treated with ether and filtered. The solid (1.40 g) thus obtained was treated with a mixture of hot MeOH, CH₂Cl₂, and ether. Filtration of the mixture gave a white solid which was washed with CH₂Cl₂ and ether, dried over P₂O₅ under vacuum, and identified as **16a**: yield 0.46 g (69%); mp 235–237 °C; ¹H NMR (DMSO-*d*₆) δ 2.00 (s, 3H), 2.03 (s, 3H), 3.17–3.20 (m, 1H), 3.37–3.41 (m, 1H), 3.45–3.50 (m, 1H), 6.29 (s, 1H), 6.37 (s, 1H); ¹³C NMR (DMSO-*d*₆) δ 18.61, 18.91, 45.14, 53.46, 113.32, 116.29, 124.00, 124.66, 133.24, 134.31, 169.20. Anal. (C₁₁H₁₇N₃O₂·0.25H₂O) C, H, N.

β-((2'-Aminophenyl)amino)-L-alanine (16b). Phenylene-1,2-diamine (0.73 g, 6.72 mmol, dried over P₂O₅ under Ar) was dissolved in dry DMF (5 mL). The solution was stirred and cooled at 0 °C under Ar while **15a** (0.58 g, 2.24 mmol) was added. After the completion of the addition, the solution was stirred at 0 °C for 2 h and at 27 °C for 2 days. Ether was then added, and the solid that separated was filtered off. Further purification of the solid by treating with a mixture of MeOH, CH₂Cl₂, and ether gave 0.44 g (62%) of **16b** (dried over P₂O₅ under vacuum): pale yellow solid; mp 175–177 °C; ¹H NMR (DMSO-*d*₆) δ 3.25–3.34 (m, 1H), 3.40–3.50 (m, 1H), 3.83–3.87 (m, 1H), 6.45–6.55 (m, 3H), 6.57–6.61 (m, 1H); ¹³C NMR (DMSO-*d*₆) δ 44.06, 52.64, 111.04, 114.34, 117.62, 118.49, 134.92, 136.66, 169.62. Anal. (C₉H₁₃N₃O₂·³/₈TsOH·³/₄H₂O) C, H, N, S.

(S)-1-(2'-Amino-2'-carboxyethyl)-1,4-dihydro-6,7-dimethylquinoxaline-2,3-dione (2a). To a suspension of **16a** (0.33 g, 1.48 mmol) in dry CH₂Cl₂ (10 mL) was added oxyl chloride (2 M in CH₂Cl₂, 0.78 mL, 1.55 mmol) slowly with a syringe at 0 °C. The resulting mixture was allowed to warm to room temperature and stirred at room temperature overnight. Some ether was added, and the solid was separated by filtration. Recrystallization of this material from a mixture of DMF, CH₂Cl₂, and ether gave 190 mg (36%) of **2a** as a hydrochloride salt: pale yellow solid; mp 215–220 °C (darkening), 240–270 °C dec; ¹H NMR (DMSO-*d*₆) δ 2.18 (s, 3H), 2.25 (s, 3H), 4.00–4.10 (dd, 1H), 4.45–4.55 (m, 1H), 5.60–4.70 (m, 1H), 6.98 (s, 1H), 7.45 (s, 1H), 8.45–9.10 (s, br, 3H), 12.02 (s, 1H); ¹³C NMR (DMSO-*d*₆) δ 18.76, 19.09, 41.21, 49.06, 115.46, 116.50, 123.56, 123.62, 131.82, 132.09, 153.29, 156.06, 168.58; [α]_D²⁰ +34.6° (c 1.4, CH₃OH). Anal. (C₁₃H₁₆N₃O₄Cl·H₂O) C, H, N.

(S)-1-(2'-Amino-2'-carboxyethyl)-1,4-dihydroquinoxaline-2,3-dione (2b). To a suspension of **16b** (145 mg, 0.46

mmol) in dry CH_2Cl_2 (5 mL) was added oxyl chloride (2 M in CH_2Cl_2 , 0.27 mL, 0.54 mmol) slowly with a syringe at 0 °C. The resulting mixture was allowed to warm to room temperature and stirred at room temperature overnight. Some ether was added, and the solid was separated by filtration. Recrystallization of this material from a mixture of DMF, CH_2Cl_2 , and ether gave 104 mg (52%) of **2b** as a hydrochloride salt: pale yellow solid; mp 195–215 °C dec; ^1H NMR (DMSO- d_6) δ 4.10–4.25 (m, 1H), 4.55–4.70 (m, 2H), 7.15–7.28 (m, 3H), 7.32–7.45 (m, 1H), 8.30–8.85 (s, br, 3H), 12.10 (s, 1H); ^{13}C NMR (DMSO- d_6) δ 41.30, 49.60, 114.40, 115.96, 123.37, 123.97, 125.81, 126.01, 153.30, 156.26, 168.44. Anal. [$\text{C}_{11}\text{H}_{12}\text{N}_3\text{O}_4\text{Cl}\cdot 0.8(\text{TsOH}\cdot\text{H}_2\text{O})$] C, H, N.

β -(2'-Amino-4',5'-dimethylphenyl)amino)-D-alanine (16c). Under Ar, **15b** (1.00 g, 3.86 mmol) was added to a solution of 4,5-dimethylphenylene-1,2-diamine (1.84 g, 13.51 mmol) in DMF (8 mL) in several portions at 0 °C with stir. The solution was allowed to warm to room temperature and stirred overnight. Ether was added, and the suspension was filtered. The solid obtained was purified by treating with $\text{MeOH}-\text{CH}_2\text{Cl}_2$ -ether and dried over P_2O_5 under vacuum, and 0.61 g (59%) of **16c** was recovered which was contaminated with $1/4\text{TsOH}$: white solid; mp 232–234 °C; ^1H NMR (DMSO- d_6) δ 2.00 (s, 3H), 2.04 (s, 3H), 3.17–3.24 (m, 1H), 3.37–3.43 (m, 1H), 3.55–3.70 (m, 1H), 6.31 (s, 1H), 6.39 (s, 1H); ^{13}C NMR (DMSO- d_6) δ 18.59, 18.90, 45.02, 66.97, 113.38, 116.37, 124.09, 124.78, 133.21, 134.25. Anal. ($\text{C}_{11}\text{H}_{17}\text{N}_3\text{O}_2\cdot 0.5\text{H}_2\text{O}$) C, H, N.

β -(2'-Aminophenyl)amino)-D-alanine (16d). A solution phenylene-1,2-diamine (0.94 g, 8.70 mmol, dried over P_2O_5 under Ar) in DMF (8 mL) was cooled to 0 °C under Ar. **15b** (0.75 g, 2.90 mmol) was added portionwise with stirring. The solution was allowed to warm to room temperature and stirred overnight at room temperature and for 8 h for 30 °C. The mixture was allowed to warm to room temperature, treated with ether, and filtered. Treatment of the solid obtained with $\text{MeOH}-\text{CH}_2\text{Cl}_2$ -ether gave 0.32 g (31%) of **16d**, containing 0.8 $\text{TsOH}\cdot\text{H}_2\text{O}$: pale yellow solid; mp 167–169 °C; ^1H NMR (DMSO- d_6) δ 3.25–3.40 (m, 1H), 3.45–3.55 (m, 1H), 3.85–3.95 (m, 1H), 6.47–6.56 (m, 3H), 6.58–6.65 (m, 1H); ^{13}C NMR (DMSO- d_6) δ 44.01, 52.60, 111.05, 114.38, 117.65, 118.52, 134.90, 136.64, 169.82. Anal. ($\text{C}_9\text{H}_{13}\text{N}_3\text{O}_2\cdot 0.8\text{TsOH}\cdot\text{H}_2\text{O}$) C, H, N, S.

(R)-1-(2'-Amino-2'-carboxyethyl)-1,4-dihydro-6,7-dimethylquinoxaline-2,3-dione (2c). To a suspension of **16c** (0.24 g, 0.90 mmol) in dry CH_2Cl_2 (10 mL) was added oxyl chloride (2 M in CH_2Cl_2 , 0.61 mL, 1.22 mmol) slowly with a syringe at 0 °C. The resulting mixture was allowed to warm to room temperature and stirred at room temperature overnight and at 28–30 °C for 6 h. More CH_2Cl_2 was added, and the solid was separated by filtration, resolidified from DMF- CH_2Cl_2 -ether, filtered, and washed with CH_2Cl_2 ; 0.13 g (43%) of **2c** was recovered as a slightly colored solid; mp 240–270 °C dec; ^1H NMR (DMSO- d_6) δ 2.19 (s, 3H), 2.26 (s, 3H), 4.06–4.10 (dd, 1H), 4.45–4.70 (m, 2H), 7.00 (s, 1H), 7.40 (s, 1H), 8.20–8.95 (s, br, 3H), 11.99 (s, 1H); ^{13}C NMR (DMSO- d_6) δ 18.72, 19.09, 41.27, 49.21, 115.38, 116.46, 123.57, 123.63, 131.72, 132.06, 153.29, 156.07, 168.53. Optical rotation, $[\alpha]_D^{20}$ –32.9° (c 1.4, CH_3OH). Anal. ($\text{C}_{13}\text{H}_{16}\text{N}_3\text{O}_4\text{Cl}\cdot 0.2\text{DMF}\cdot 0.5\text{H}_2\text{O}$) C, H, N.

(R)-1-(2'-Amino-2'-carboxyethyl)-1,4-dihydroquinoxaline-2,3-dione (2d). To a suspension of **16d** (200 mg, 0.57 mmol) in dry CH_2Cl_2 (8 mL) was added oxyl chloride (2 M in CH_2Cl_2 , 0.3 mL, 0.60 mmol) slowly with a syringe at 0 °C. The resulting mixture was allowed to warm to room temperature and stirred at room temperature overnight and at ca. 30 °C for 3 h. After the mixture was allowed to cool to room temperature, more CH_2Cl_2 was added, and the solid produced was separated by filtration. Recrystallization of this material from a mixture of DMF- CH_2Cl_2 -ether gave 136 mg (50%) of **2d** as a pale yellow solid; mp 195–215 °C dec; ^1H NMR (DMSO- d_6) δ 4.05–4.16 (m, 1H), 4.55–4.68 (m, 2H), 7.15–7.27 (m, 3H), 7.45–7.55 (m, 1H), 8.35–8.85 (s, br, 3H), 12.14 (s, 1H); ^{13}C NMR (DMSO- d_6) δ 41.32, 49.36, 114.56, 116.00, 123.40, 123.95, 125.84, 126.01, 153.28, 156.20, 168.46. Anal. [$\text{C}_{11}\text{H}_{12}\text{N}_3\text{O}_4\text{Cl}\cdot 0.5(\text{TsOH}\cdot\text{H}_2\text{O})$] C, H, N, S.

Biological Method. The interaction of various quinoxalinedione analogs with AMPA and kainate receptors was evaluated by determining their ability to inhibit the binding of [^3H]AMPA²² and [^3H]kainate²³ to rat brain homogenates. [^3H]AMPA binding was determined in the presence of 100 μM potassium thiocyanate, which has been shown to enhance specific binding.²⁴ Functional agonist activity was determined by the ability to stimulate [^3H]norepinephrine release in a manner that was inhibited by DNQX. Functional antagonist activity was determined by the ability to inhibit AMPA-stimulated [^3H]norepinephrine release from hippocampal slices. Briefly, slices from hippocampus are incubated in the presence of [^3H]norepinephrine, washed to remove extracellular [^3H]norepinephrine that has not been taken up into the slices, and then superfused with compounds to be tested for ability to release [^3H]norepinephrine.²⁵ AMPA and kainate in the presence of cyclothiazide (an inhibitor of receptor desensitization) reliably release [^3H]norepinephrine from the slices (Figure 2). These responses are blocked by DNQX (Figure 3). The specificity and reliability of this procedure have been well documented.

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