Synthesis and Biological Evaluation of Analogues of 7-Chloro-4,5-dihydro-4-oxo-8-(1,2,4-triazol-4-yl)-1,2,4-triazolo[1,5-*a*]quinoxaline-2-carboxylic Acid (TQX-173) as Novel Selective AMPA Receptor Antagonists

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In recent papers (Catarzi, D.; et al. *J. Med. Chem.* **2000**, *43*, 3824−3826; **2001**, *44*, 3157−3165) we reported chemical and biological studies on 4,5-dihydro-4-oxo-1,2,4-triazolo[1,5-*a*]-quinoxaline-2-carboxylates (TQXs) bearing different nitrogen-containing heterocycles at position-8. In particular, from these studies it emerged that both the 7-chloro-4,5-dihydro-4-oxo-8-(1,2,4-triazol-4-yl)-1,2,4-triazolo[1,5-*a*] quinoxaline-2-carboxylic acid TQX-173 (compound **B**) and its corresponding ethyl ester (compound **A**) were the most active and selective compounds of this series. In pursuing our investigation on the structure−activity relationships of these TQX derivatives, different electron-withdrawing groups (CF_3, NO_2) were introduced at position 7 on the TQX ring system, replacing the 7-chloro substituent of **B** and of other selected 8-heteroaryltriazoloquinoxaline-2-carboxylates previously described. All the newly synthesized compounds were biologically evaluated for their binding at the Gly/NMDA, AMPA, and KA high-affinity receptors. Gly/NMDA binding assays were performed to assess the selectivity of the reported compounds toward the AMPA receptor. Compounds endowed with micromolar binding affinity for the KA high-affinity binding site were also evaluated for their binding at the KA low-affinity receptor. Some selected compounds were also tested for their functional antagonist activity at the AMPA and NMDA receptor-ion channel complex. The results obtained in this study have pointed out that 4,5-dihydro-7-nitro-4-oxo-8-(3-carboxypyrrol-1-yl)-1,2,4-triazolo[1,5-*a*]quinoxaline-2-carboxylic acid (**9b**) and its corresponding ethyl ester (**9a**) are the most potent and selective AMPA receptor antagonists reported to date among the TQX series.

Introduction

Glutamate (Glu) is the major excitatory neurotransmitter in the central nervous system (CNS), where it is involved in the physiological regulation of processes such as learning, memory, and synaptic plasticity. On the other hand, glutamatergic hyperactivity may lead to neurotoxicity. In fact, excessive endogenous Glu is implicated in a number of acute and chronic neurodegenerative pathologies such as cerebral ischaemia, epilepsy, amiotrophic lateral sclerosis, and Parkinson's diseases.[1]

Glu activates specific receptors that belong to the classes of metabotropic receptors (mGluRs, coupled to G-protein) and ionotropic receptors (iGluRs, ligand-gated ion channel), the latter consisting of two primary families: *N*-methyl-D-aspartic acid (NMDA) receptor family and non-NMDA receptor family. The non-NMDA family is composed of the kainic acid (KA) receptor and the (*R*,*S*)-2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)-propionic acid (AMPA) receptor according to their preferential synthetic agonists.[1,2] The physiological and pathological roles of both AMPA and NMDA receptor-ion channel complex are well understood; on the contrary, the physiological function of the KA receptor family, comprising the low-affinity binding site (GluR5-7 subunits) and the high-affinity one (KA1-2 subunits), is still an open question.[1,3−5]

The well-known physiological, but also pathological, role of Glu has resulted in a great interest for the development of Glu receptor (GluR) ligands as research tools and potential therapeutic agents.[1]

Several AMPA receptor antagonists have been reported in the literature and show promise in terms of their therapeutic potential as neuroprotective agents for the treatment and prevention of acute and chronic neurological disorders.[1,3−4,6−11]

However, only a limited number of KA receptor antagonists have been developed, which, in general, have shown mixed AMPA/KA inhibitor activity.[1,3−4,6] This is due to the fact that structural similarities and differences between these two diverse receptors are still unknown. For this reason, there are some difficulties in pharmacologically differentiating AMPA and KA receptors. To our knowledge, only the 5-nitro-6,7,8,9-tetrahydro-1*H*-benz[*g*]indole-2,3-dione 3-oxime NS-102 shows a high affinity and selectivity for the low-affinity KA receptor binding site.[1,12] It has been reported that compounds showing high affinity for both AMPA and KA low-affinity binding sites are more potent anticonvulsants than AMPA receptor selective drugs.[13] Thus,

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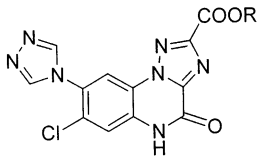
Chart 1



A : R = Et
B : R = H **(TQX-173)**

KA and AMPA receptors can be considered as potential targets for therapeutic prevention of epileptic seizures.[11,13]

Moreover, an alternative approach to antagonize the overstimulation of postsynaptic iGluRs by excessive endogenous Glu is represented by the use of non-competitive NMDA antagonists acting at the strychnine-insensitive glycine site (Gly/NMDA) on the NMDA receptor. In fact, glycine (Gly) is a necessary coagonist of Glu and exerts a primary role in the activation of the NMDA receptor by interacting with its allosteric modulatory site.[14,15] Thus, blockade of the Gly/NMDA receptor by antagonists results in a complete inhibition of NMDA-mediated neurodegenerative effects.[1,15−19]

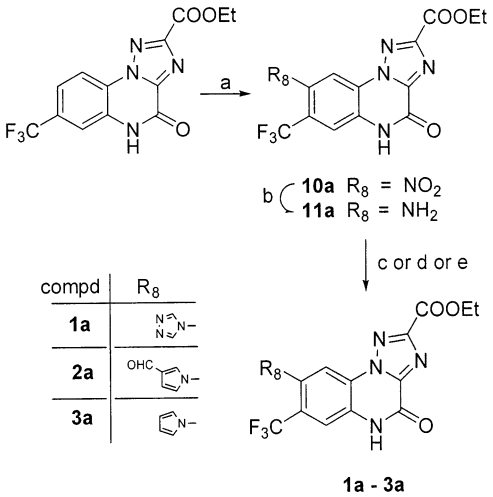
Accordingly, there has been a growing interest in competitive and non-competitive iGluR antagonists due to their promising therapeutic potential.

A non-selective blockade of both Gly/NMDA and AMPA/KA receptors was shown by quinoxalinedione and heterocyclic-fused quinoxalinone antagonists.[15,20−24] Extensive structure−activity relationship (SAR) studies on these derivatives have pointed out not only some important common structural requirements for anchoring at both receptor sites[7,9,21−22] but also some different features that are important in distinguishing the AMPA/KA receptors from the Gly/NMDA one.[6−9,21−22,25−28] In particular, introduction of suitable substituents on precise positions on the benzofused moiety of these antagonists yielded selective and competitive blockade of the AMPA/KA receptor.[6−7,9,21−22,26−28]

In the course of our efforts to find novel competitive and non-competitive iGluR antagonists,[29−37] we have published works that report the synthesis and biological studies on 4,5-dihydro-4-oxo-1,2,4-triazolo[1,5-*a*]quinoxaline-2-carboxylates (TQXs) bearing different nitrogen-containing heterocycles at position-8.[34,36] These previous studies have well-established that the presence of a N^3-nitrogen-containing heterocycle at position-8 of the TQX framework is an essential feature for potent and selective AMPA receptor antagonists. In particular, it has emerged that both the 7-chloro-4,5-dihydro-4-oxo-8-(1,2,4-triazol-4-yl)-1,2,4-triazolo[1,5-*a*]quinoxaline-2-carboxylic acid TQX-173 (compound **B**) and its corresponding ethyl ester (compound **A**) (Chart 1) are the most active and selective compounds among this series.[34,36]

In pursuing our studies on the SAR of these TQX derivatives and in order to develop new AMPA receptor antagonists with increased potency and selectivity, we have introduced some modifications on the tricyclic system, in particular at position-7. In fact, on the basis of a reported pharmacophore model of the AMPA receptor,[7−9] different electron-withdrawing groups (CF_3, NO_2) were introduced at position-7 on the herein reported TQX compounds, replacing the 7-chloro substituent of **B** and of other selected 8-heteroaryltriazoloquinoxaline-2-carboxylates previously described.

Scheme 1[a]



[a] Reagents: (a) 90% HNO_3; (b) iron, glacial AcOH; (c) $(OHC-NH)_2$, anhydrous pyridine, Me_3SiCl, Et_3N; (d) 2,5-dimethoxy-3-tetrahydrofurancarboxyaldehyde, glacial AcOH; (e) 2,5-diethoxytetrahydrofuran, glacial AcOH.

All of the newly synthesized compounds were biologically evaluated for their binding at the Gly/NMDA, AMPA, and KA high-affinity receptors. Gly/NMDA and KA binding assays were performed to assess the selectivity of the reported compounds toward the AMPA receptor. Compounds endowed with micromolar binding affinity for the KA high-affinity binding site were also evaluated for their binding at the KA low-affinity receptor.

Some selected compounds were also tested for their functional antagonist activity at the AMPA and NMDA receptor-ion channel complex.

Chemistry

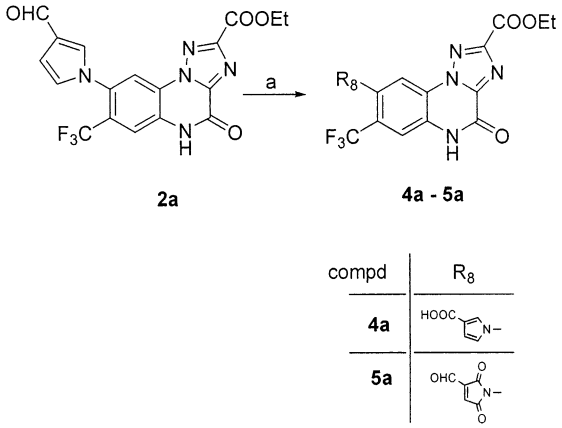
The ethyl 8-substituted-4,5-dihydro-4-oxo-1,2,4-triazolo[1,5-*a*]quinoxaline-2-carboxylates **1a**−**13a** were prepared following two different synthetic strategies illustrated in Schemes 1−4.

The previously reported ethyl 4,5-dihydro-4-oxo-7-trifluoromethyl-1,2,4-triazolo[1,5-*a*]quinoxaline-2-carboxylate[33] was regioselectively nitrated at position-8 to yield **10a**, which was reduced to the corresponding 8-amino derivative **11a** (Scheme 1). By reacting **11a** with diformylhydrazine, the 8-(1,2,4-triazol-4-yl) ester **1a** was prepared. The 8-(3-formylpyrrol-1-yl) ester **2a** and the 8-(pyrrol-1-yl) ester **3a** were obtained by reacting **11a** with either 2,5-dimethoxy-3-tetrahydrofurancarboxaldehyde or 2,5-diethoxytetrahydrofuran, respectively.

Oxidation of the 8-(3-formylpyrrol-1-yl) derivative **2a** with potassium permanganate yielded a mixture of the 8-(3-carboxypyrrol-1-yl) ester **4a** and the 8-(3-formyl-2,5-dioxopyrrol-1-yl) **5a** (Scheme 2), which were separated by using silica gel column chromatography.

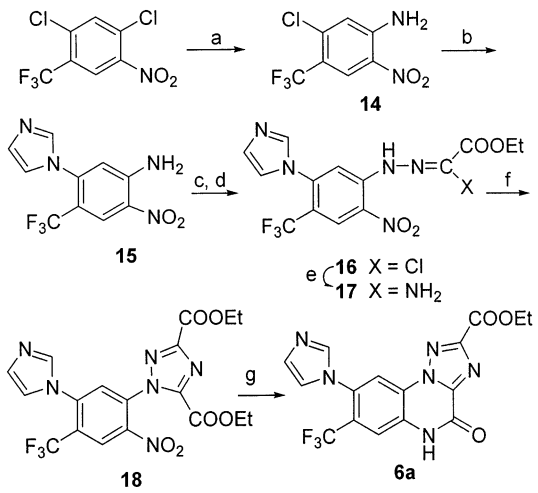
By reacting the commercially available 2,4-dichloro-5-nitrobenzotrifluoride with ammonia, the 5-chloro-2-nitro-4-trifluoromethylaniline **14**[38] was isolated (Scheme

Scheme 2[a]



[a] Reagents: (a) $KMnO_4$, acetone/water 1:1.

Scheme 3[a]



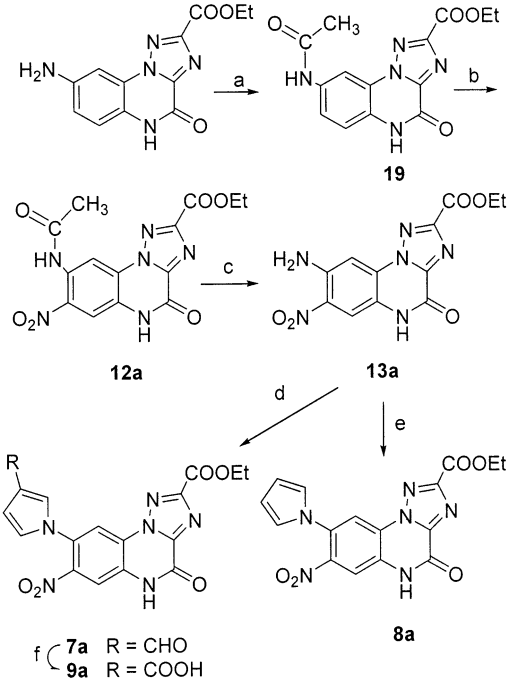
[a] Reagents: (a) NH_3(gas), 2-methoxyethanol; (b) imidazole, KOH, anhydrous DMF; (c) $NaNO_2$/conc H_2SO_4, H_2O; (d) CH_3CO-CHClCOOEt, MeOH; (e) NH_3 (gas), anhydrous dioxane; (f) ClCO-COOEt, anhydrous toluene; (g) iron powder, glacial AcOH.

3). Reaction of **14** with imidazole in alkaline conditions yielded the corresponding 5-(imidazol-1-yl)-2-nitro-4-trifluoromethylaniline **15**,[39] which was transformed into its diazonium sulfate by diazotization with $NaNO_2$ in H_2SO_4. The diazonium salt of **15** was reacted with ethyl 2-chloro-3-oxobutanoate to yield the N^2-chloroacetate **16**, which was transformed with ammonia into its corresponding N^2-oxamidrazonate **17**. By reacting **17** with ethyloxalyl chloride, the diethyl 1-[5-(imidazol-1-yl)-2-nitro-4-trifluoromethylphenyl]-1,2,4-triazole-3,5-dicarboxylate **18** was directly obtained. Reduction of the nitro group of **18** and contemporary cyclization afforded the tricyclic ester **6a**.

Reaction of the ethyl 8-amino-4,5-dihydro-4-oxo-1,2,4-triazolo[1,5-*a*]quinoxaline-2-carboxylate[36] (Scheme 4) with acetic anhydride yielded the corresponding 8-acetylamino derivative **19**, which was nitrated at position-7 with 90% HNO_3 to afford **12a**. Acidic hydrolysis of the acetyl group of **12a** yielded the 8-amino-7-nitro derivative **13a**.

By reacting **13a** with either 2,5-dimethoxy-3-tetrahydrofurancarboxaldehyde or 2,5-diethoxytetrahydrofuran in glacial AcOH, the 8-(3-formylpyrrol-1-yl) **7a** and the 8-(pyrrol-1-yl) **8a** esters were obtained, respectively.

Scheme 4[a]



[a] Reagents: (a) Ac_2O, glacial AcOH; (b) 90% HNO_3; (c) 6 N HCl, EtOH; (d) 2,5-dimethoxy-3-tetrahydrofurancarboxyaldehyde, glacial AcOH; (e) 2,5-diethoxytetrahydrofuran, glacial AcOH; (f) $KMnO_4$, acetone/water.

Oxidation of compound **7a** with potassium permanganate afforded the 8-(3-carboxypyrrol-1-yl) derivative **9a**.

Finally, the hydrolysis of the esters **1a-4a** and **6a-13a** to yield the corresponding 2-carboxylic acids **1b-4b** and **6b-13b** is depicted in Scheme 5.

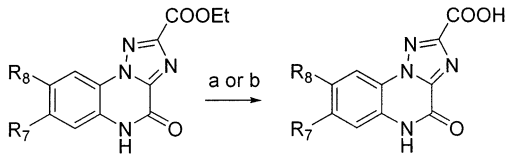
Results and Discussion

The triazoloquinoxalines **1a,b**−**4a,b**, **5a**, and **6a,b**−**13a,b** together with NBQX (2,3-dihydroxy-6-nitro-7-sulfamoylbenzo[*f*]quinoxaline) and DCKA (5,7-dichlorokinurenic acid) as standard compounds, were tested for their ability to displace tritiated AMPA and Gly from their specific binding in rat cortical membranes. High-affinity KA binding assays were also performed. The binding results are shown in Table 1 together with those of previously reported 2-carboxylic acid derivative **B** and its corresponding ester **A** included as reference compounds.[34,36] Some selected compounds (**1b**, **2b**, **4b**, **7b**, **8b**) were also evaluated for their binding at the KA low-affinity receptor, and the results are shown in Table 2.

Since most of the newly synthesized compounds were AMPA vs KA receptor non-selective, with the exception of **1a,b**, **2a**, **3a**, **4b**, **6a,b**, **9a,b**, and **10a** together with the reference **B** (K_i KA vs AMPA ≥ 80), only AMPA vs Gly/NMDA receptor selectivity of these TQX derivatives has been considered in the discussion of the herein reported results.

In general, the affinities of the TQX derivatives **1a,b**−**4a,b**, **5a,** and **6a,b**−**13a,b** for the Gly/NMDA receptor are lower than those for the AMPA receptor, confirming previously reported data. In fact, the binding data shown in Table 1 indicate that replacement of the 7-chloro substituent of some selected previously reported 8-heteroaryl TQX derivatives,[34,36] with a trifluoromethyl or a nitro group, has produced new highly potent and selective AMPA receptor antagonists.

Scheme 5[a]



1a - 4a, 6a - 13a **1b - 4b, 6b -13b**

compd	R_7	R_8
1a, 1b	CF_3	
2a, 2b	CF_3	
3a, 3b	CF_3	
4a, 4b	CF_3	
6a, 6b	CF_3	
7a, 7b	NO_2	
8a, 8b	NO_2	
9a, 9b	NO_2	
10a, 10b	CF_3	NO_2
11a, 11b	CF_3	NH_2
12a, 12b	NO_2	$NHCOCH_3$
13a, 13b	NO_2	NH_2

[a] Reagents: (a) 0.8 N NaOH/EtOH, 6 N HCl or glacial AcOH; (b) HCl conc.

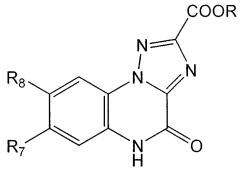
In particular, the binding data indicate that all the 2-carboxylic acids **1b**−**4b** and **6b**−**9b** are, in general, more active than their corresponding ethyl esters **1a**−**4a** and **6a**−**9a** at the AMPA receptor. In accordance with our previously reported results,[33−34,36] the difference between the binding affinity of the esters with respect to the corresponding acids is generally dependent on the nature of the 8-heteroaryl substituent. In fact, the 2-carboxylic acid derivatives bearing at position-8 a N^3-nitrogen-containing heterocycle (**1b** and **6b**), a 3-formyl-substituted heterocycle (**2b** and **7b**), or a 3-carboxy-substituted heterocycle (**9b**) are only 2−3-fold more active at the AMPA receptor than their corresponding esters (**1a**, **6a**, **2a**, **7a**, and **9a**). On the contrary, the 2-carboxylic acid **3b** and **8b**, bearing a 8-heterocyclic ring lacking the 3-formyl group, are about 10−15-fold more active than their corresponding esters **3a** and **8a**.

Thus, these results confirm that the presence of a free carboxylic group at position-2 is not an essential feature for the anchoring to the AMPA receptor when the 8-heteroaryl substituent bears at position-3 a proton-acceptor atom/group.[34] Indeed, the N^3-nitrogen atom, the 3-formyl, or the 3-carboxy group could act as proton acceptors in a hydrogen bond with a specific receptor site, thus reinforcing the receptor−ligand interaction.

It has to be noted that the 2-carboxylic acid **4b**, bearing at position-8 a 3-carboxylic acid substituted heterocycle, is 10-fold more active than its corresponding ester **4a**. This is an unexpected result on the basis of the above-reported data, because the carboxylic acid function on the 8-heterocycle substituent is a powerful proton acceptor group.

Replacement of the 7-chloro substituent of **B** and its corresponding 2-carboxylic ester **A** with a trifluoro-

Table 1. Binding Affinity at AMPA, Gly/NMDA, and KA High-Affinity Receptors of TQX Derivatives[a]



Compd	R	R_7	R_8	Ki(μM)[b] or I%[c] [^{3}H]AMPA	Ki(μM)[b] or I%[c] [^{3}H]Gly	IC_{50}(μM)[d] or I%[c] [^{3}H]KA
1a	Et	CF_3		0.2 ± 0.01	42%	92 ± 16
1b	H	CF_3		0.095 ± 0.01	3.6 ± 0.25	9.6 ± 0.6
2a	Et	CF_3		0.37 ± 0.03	71 ± 4	31.2 ± 4.2
2b	H	CF_3		0.12 ± 0.01	2.6 ± 0.7	5.6 ± 0.4
3a	Et	CF_3		2.2 ± 0.4	75 ± 8	5%
3b	H	CF_3		0.28 ± 0.02	8.3 ± 2.1	18.1 ± 1.7
4a	Et	CF_3		0.35 ± 0.02	33%	12.9 ± 1.3
4b	H	CF_3		0.037 ± 0.005	35 ± 3	5.4 ± 0.4
5a	Et	CF_3		12.8 ± 2.1	10.9 ± 1.3	42 ± 7
6a	Et	CF_3		0.35 ± 0.02	54 ± 9	71 ± 7
6b	H	CF_3		0.12 ± 0.01	34 ± 5	13 ± 1
7a	Et	NO_2		0.65 ± 0.04	28%	44.4 ± 7.1
7b	H	NO_2		0.18 ± 0.02	43%	8.2 ± 1.0
8a	Et	NO_2		1.4 ± 0.14	84 ± 7	41 ± 6
8b	H	NO_2		0.088 ± 0.015	65 ± 5.4	5.1 ± 0.5
9a	Et	NO_2		0.068 ± 0.005	16%	38%
9b	H	NO_2		0.019 ± 0.002	7%	17 ± 2
10a	Et	CF_3	NO_2	9.0 ± 1.0	62 ± 12	24%
10b	H	CF_3	NO_2	2.35 ± 0.35	4.4 ± 1.0	43 ± 7
11a	Et	CF_3	NH_2	5.5 ± 1.3	5.5 ± 0.9	45%
11b	H	CF_3	NH_2	1.2 ± 0.15	1.5 ± 0.6	24 ± 4.6
12a	Et	NO_2	$NHCOCH_3$	6.5 ± 0.7	85 ± 14	41 ± 5.8
12b	H	NO_2	$NHCOCH_3$	1.14 ± 0.2	63 ± 6	22.2 ± 1.4
13a	Et	NO_2	NH_2	1.2 ± 0.2	3.7 ± 0.8	69 ± 5
13b	H	NO_2	NH_2	0.7 ± 0.08	2.7 ± 0.05	20 ± 2
A[e]	Et	Cl		0.70 ± 0.13	15%	42%
B[e]	H	Cl		0.14 ± 0.02	33.5 ± 5.3	11.3 ± 1.3
NBQX	-	-	-	0.07 ± 0.06	3%	7.0 ± 1.1
DCKA	-	-	-	5%	0.09 ± 0.02	8%

[a] The tested compounds were dissolved in DMSO and then diluted with the appropriate buffer. [b] Inhibition constant (K_i) values were means ± SEM of three or four separate determinations in triplicate. [c] Percentage of inhibition (*I*%) of specific binding at 100 μM concentration. [d] Concentration necessary for 50% inhibition (IC_{50}). The IC_{50} values were means ± SEM of three or four separate determinations in triplicate.[34]

Table 2. Binding Affinity at KA Low-Affinity Binding Site of Some Selected TQX Derivatives[a]

compd	IC_{50} (μM)[b] [^{3}H]KA	IC_{50} ratio[c]	compd	IC_{50} (μM)[b] [^{3}H]KA	IC_{50} ratio[c]
1b	1.8 ± 0.2	0.19	**7b**	1.2 ± 0.09	0.15
2b	1.0 ± 0.2	0.18	**8b**	1.7 ± 0.4	0.33
4b	2.6 ± 0.3	0.48	NBQX	1.5 ± 0.07	0.21

[a] The tested compounds were dissolved in DMSO and then diluted with the appropriate buffer. [b] Concentration necessary for 50% inhibition (IC_{50}). The IC_{50} values were means ± SEM of three or four separate determinations in triplicate. [c] IC_{50} at KA low-affinity receptor site/IC_{50} at KA high-affinity receptor site (see Table 1).

methyl moiety produces compounds **1b** and **1a**, which show comparable and 3.5-fold increased AMPA binding affinities compared to those of the reference compounds, respectively. While compound **1a** is one of the most AMPA-selective compounds among the TQX series, its corresponding acid **1b** is much less selective than **B**, due to its 10-fold increased binding affinity at the Gly/NMDA receptor. The high AMPA selectivity of compound **1a** can be considered an exception. In fact, as discussed below, the contemporary presence of the 2-carboxylic acid function together with the 7-trifluoromethyl substituent on the TQX framework produces an ameliorated interaction with both the Gly/NMDA and AMPA receptor sites, thus positively affecting binding affinities to a small degree, but not selectivity.

Maintaining the 7-trifluoromethyl substitution, the 8-(1,2,4-triazol-4-yl) moiety was replaced by the 8-(3-formylpyrrol-1-yl) or 8-(imidazol-1-yl) moiety, giving compounds **2a,b** and **6a,b**, respectively, which have AMPA binding affinity values comparable to those of **1a,b**. It has to be noted that, while compounds **1b** and **2b**, as discussed above, show a low AMPA selectivity, **6b** is one of the most AMPA-selective 2-carboxylic acid derivatives among this series. The high AMPA selectivity of **6b** can be explained by hypothesizing that the 8-(imidazol-1-yl) moiety is not well-tolerated by the Gly/NMDA receptor site.

Elimination of the 3-formyl group from the 8-substituent of compounds **2a,b** gives the corresponding 8-(pyrrol-1-yl) derivatives **3a,b**, which are 6- and 2,3-fold less active at the AMPA receptor than their parents **2a,b**.

These data confirm that the N^3-nitrogen atom or the 3-formyl group can act as a proton acceptor in a hydrogen bond with a receptor site, thus reinforcing the receptor−ligand interaction (compare **1a,b**, **2a,b**, and **6a,b** to **3a,b**, respectively).

Replacement of the 3-formyl group on the 8-substituent of **2a,b** with a free carboxylic group gives compounds **4a,b**, which are equiactive and about 3-fold more active at the AMPA receptor than **2a,b**, respectively. More importantly, an increased selectivity toward the AMPA receptor was observed. In fact, the 2-carboxylic acid **4b** is one of the most potent and selective antagonists among the TQX series. This result can be explained hypothesizing that a 3-anionic carboxylate residue on the 8-substituent is able to engage a strong interaction with a cationic proton donor site of the AMPA receptor, but it is not well-tolerated by the Gly/NMDA receptor site. This hypothesis is supported by the high AMPA vs Gly/NMDA selectivity of the 2-ethyl carboxylate **4a**.

On the contrary, oxidation of both 2- and 5-positions of the 8-(3-formylpyrrol-1-yl) moiety of **2a** gives compound **5a**, which is about 30-fold less active at the AMPA receptor than **2a**, indicating that an effect of steric hindrance may be operative at the 8-position. In fact, due to the steric hindrance exerted by the 2,5-dioxo function, the 8-substituent of **5a** is probably twisted out of the plane of the TQX framework, owing to an unfavorable steric interaction at this level. These data could suggest that in the AMPA receptor both sites that accommodate, respectively, the 8-heteroaryl substituent and the tricyclic ring system of AMPA antagonists such as TQXs lie in the same plane.

No data about the replacement of the 7-chloro substituent of **B** with a nitro group are available. In fact, the synthesis of the 7-nitro analogue of **A**, following the same procedures used to prepare **1a**, was not achieved, probably because of the very low reactivity of the 8-amino group of the starting compound **13a**. Other synthetic strategies (data not published) have not given the desired compound either.

Replacement of the 7-trifluoromethyl group of **2a,b** with a nitro group gives compounds **7a,b**, which are about equiactive at the AMPA receptor as their parents **2a,b**. Both the 2-carboxylic ester **7a** and its corresponding 2-carboxylic acid **7b**, due to the inactivity at the Gly/NMDA receptor, were much more selective toward the AMPA receptor than **2a** and **2b**, respectively. Increased AMPA activity and selectivity was observed when the nitro group replaced the trifluoromethyl substituent of the 2-carboxylic acid **3b** to give **8b**. On the contrary, the same modification on the 2-carboxylic ester **3a** unexpectedly produced only a 1.5−2-fold increase of AMPA binding affinity and selectivity (compare **8a** to **3a**).

These data indicate that the presence of the mesomeric electron-withdrawing nitro group at the 7-position of our TQX derivatives is an important feature for potent and selective AMPA receptor antagonists, independent from both the nature of the 8-heteroaryl substituent and the presence of the 2-carboxylic acid function. In fact, the 8-(pyrrol-1-yl)-substituted derivative **8b**, although bearing both a N^3-nitrogen-lacking heterocycle at position-8 and a carboxylic acid function at position-2, is one of the most AMPA receptor selective compounds among this series.

Taking into consideration the positive effect exerted on AMPA binding affinity and selectivity by both the 7-nitro substituent and the 8-(3-carboxypyrrol-1-yl) moiety, compounds **9a,b** were rationally designed. The binding results of these compounds showed that the contemporary presence on the TQX framework of these two profitable groups is optimal to achieve significant AMPA binding affinity and selectivity. In fact, compounds **9a,b** are not only highly selective for AMPA vs Gly/NMDA (selectivity ratio > 1500) but also AMPA vs KA (selectivity ratio > 900). Thus, **9a** and **9b** are the most potent and selective AMPA receptor antagonists among the TQX series.

In summary, replacement of the 7-chlorine atom of the analogues of compound **B** with a powerful electron-withdrawing substituent such as trifluoromethyl or nitro group yields, in general, AMPA receptor antagonists more potent than the 7-chloro substituted ones. Moreover, the presence of the strong electron-withdraw-

ing nitro group also enhances the AMPA selectivity of these derivatives. In fact, in accordance with the reported AMPA pharmacophore model,[6−9] a nitro substitution pattern at this level is, in general, unfavorable at the Gly/NMDA site but preferable for the AMPA receptor.

Replacement of the 8-heteroaryl moiety of the 7-trifluoromethyl-substituted derivatives **1a,b**−**4a,b**, **5a**, and **6a,b** with a nitro or amino group gives compounds **10a,b** and **11a,b**, respectively, which show in general a decreased AMPA binding affinity.

A similar effect exerted by both the 8-nitro and the 8-amino groups on the AMPA binding affinity was also previously observed.[36] The 8-nitro substituent, due to its electron-withdrawing effect, could increase the NH lactam acidity, thus reinforcing the essential hydrogen-bond receptor−ligand interaction at this level. On the other hand, the 8-amino group could act as a proton acceptor in a hydrogen-bond interaction with a receptor site. The positive effect of the 8-amino group is also exerted on the Gly/NMDA binding affinity. In fact, the 8-amino derivative **11a** is 11-fold more active than the corresponding 8-nitro compound **10a**. The profitable effect of the 8-amino group is confirmed by the binding activity of compound **13b** that, although bearing a 7-nitro substituent that is not well-tolerated by the Gly/NMDA receptor site, has a comparable binding affinity to that of the 7-trifluoromethyl-substituted derivatives **11b**.

However, when the 7-trifluoromethyl group of **11a** was replaced by a nitro group, compound **13a** was obtained, showing about a 3−4-fold increased AMPA binding affinity and selectivity.

Compound **12a**, which is the 8-*N*-acetyl derivative of **13a**, is about 6-fold less active at the AMPA receptor than **13a**. On the contrary, its corresponding 2-carboxylic acid **12b** is equiactive to **13b**, but more AMPA selective, due to its low Gly/NMDA receptor affinity.

Finally, the triazoloquinoxalines **1a,b**−**4a,b**, **5a**, **6a,b**−**13a,b** are, in general, active in the high micromolar range at the high-affinity KA receptor, with the exceptions being the 2-carboxylic acids **1b**, **2b**, **4b**, **7b**, and **8b**, which display IC_{50} values in the range 5−10 μM. These data confirm that the presence of a free carboxylic group at position-2 seems to be of paramount importance for KA receptor−ligand interaction.

All the most active TQX derivatives at the KA high-affinity binding site (**1b**, **2b**, **4b**, **7b**, and **8b**) together with NBQX as reference compound were also evaluated for their binding at the KA low-affinity binding site.[39] While the well-known quinoxalinedione-like antagonist NBQX shows only a slight preference for the KA-low affinity site with respect to the KA high-affinity one (low/high-affinity IC_{50} ratio = 0.21), so far, only the isatin-oxime NS-102 is reported to selectively interact with the KA low-affinity site (IC_{50} ratio < 0.06).[12,40] In our experiments (Table 2) the IC_{50} ratio of the tested compounds **1b**, **2b**, **4b**, **7b**, and **8b** (0.14−0.48) are in the range of the quinoxalinedione-like antagonist NBQX.

To correlate the binding data with functional AMPA receptor activity, some of the herein reported compounds, endowed with high affinity for AMPA receptor, were tested for their ability to inhibit both AMPA-induced depolarization in mouse cortical wedge preparations[41] (compounds **1a,b**, **2a,b**, **3b**, **4b**, **7b**, and **8b**) and [^{3}H]norepinephrine release from rat hippocampal synaptosomes (compounds **1b**, **2b**, **3b**, **4b**, **7b**, and **8b**). In fact, since it has been reported that AMPA receptor activation induces norepinephrine release from hippocampal nerve endings,[42] the same compounds were assayed for their ability to antagonize this action in rat [^{3}H]norepinephrine-preloaded hippocampal synaptosomes. Moreover, functional antagonism at the NMDA receptor-ion channel complex was also evaluated by the ability of some selected TQX derivatives to inhibit both the NMDA-induced depolarization in mouse cortical wedge preparations (compounds **1a,b**, **2a,b**, **3b**, **4b**, **7b**, and **8b**) and the binding of the channel-blocking agent [^{3}H]-(+)-MK-801((+)-5-methyl-10,11-dihydro-5*H*-benzo[*a*,*d*]cyclohepten-5,10-imine)[25,43−44] in rat cortical membranes incubated with 10 μM glutamate and 0.1 μM glycine. The results obtained in the functional studies on the newly synthesized derivatives, together with those of compound **B**, NBQX, and DCKA as reference compounds, are listed in Table 3. All the tested compounds show an antagonistic profile and inhibit AMPA and NMDA responses in a reversible manner. The results obtained in the electrophysiological assays indicate that the inhibitory actions of **1a,b**, **2a,b**, **3b**, **4b**, **7b**, and **8b** on depolarization induced by 5 μM AMPA are much higher than those on NMDA-evoked response, confirming that these derivatives are more potent antagonists at the AMPA than the Gly/NMDA receptor. Moreover, compounds **1b**−**4b** and **8b** are more potent AMPA receptor antagonists than the reference **B**.

Table 3. Functional Antagonism (IC_{50}, μM) at AMPA and NMDA Receptors of Some Selected TQX Derivatives

compd	AMPA[a]	NMDA[a]	[^{3}H]NE release[b]	[^{3}H]-(+)-MK-801[c]
1a	12 ± 2	>150	NT	NT
1b	0.8 ± 0. 1	60 ± 5	5.8 ± 1	29 ± 3
2a	7.8 ± 0.9	[d]	NT	NT
2b	1.0 ± 0.15	50 ± 4	8.1 ± 0.5	11 ± 0.8
3b	1.5 ± 0.2	80 ± 9	11.0 ± 1.8	12.7 ± 0.7
4b	1.3 ± 0.2	60 ± 15	7.5 ± 0.6	NT
7b	3.1 ± 0.4	52 ± 5	12 ± 2.5	NT
8b	1.0 ± 0.2	50 ± 8	19.0 ± 3.0	NT
B[e]	2.3 ± 0.4	46 ± 4	28.0 ± 3.1	68 ± 4
NBQX	0.2 ± 0.02	[d]	4.0 ± 0.7	NT
DCKA	52 ± 11	4.7 ± 0.9	NT	NT

[a] Concentration necessary for 50% inhibition (IC_{50}) of depolarization induced by 5 μM *S*-AMPA or NMDA in mouse cortical wedge preparation. The IC_{50} values were mens ± SEM of three separate determinations. [b] Concentration that inhibits 50% *S*-AMPA-stimulated (50 μM) [^{3}H]noradrenaline release (IC_{50}) from rat hippocampal synaptosomes. *S*-AMPA stimulatory action was assessed in the presence of 50 μM cyclothiazide; data represent the means ± SEM of three separate determinations in duplicate. [c] Concentration giving 50% inhibition of stimulated [^{3}H]-(+)-MK-801 binding: all assays were carried out in the presence of 10 μM Glu and 0.1 μM Gly. The results were calculated from three or four separate determinations in triplicate. [d] At 10 μM concentration, the inhibition was not significant. [e] Reference 34.

The IC_{50} values to inhibit AMPA-stimulated [^{3}H]-norepinephrine release were considerably higher than those effective on cortical depolarization. This is probably due to the fact that a high concentration of S-AMPA (50μM) is necessary to obtain a measurable [^{3}H]catecholamine efflux from synaptosomes.

Moreover, **1b**, **2b**, and **3b**, being the most potent compounds at the Gly/NMDA receptor, show IC_{50} values

for glutamate stimulated [^{3}H]-(+)-MK-801 binding that correlate with their K_i values on [^{3}H]glycine binding assays.

In conclusion, this study has confirmed that replacement of the 7-chloro atom of compound **B** and of other 8-heterocyclic-substituted TQX derivatives previously reported with electron-withdrawing substituents more powerful than a chlorine atom has produced some potent and selective AMPA receptor antagonists. Moreover, this study has pointed out that the presence of a free carboxylate function on the 8-heteroaryl substituent could be of paramount importance to increase AMPA receptor affinity and selectivity. In fact, it has emerged that 4,5-dihydro-7-nitro-4-oxo-8-(3-carboxypyrrol-1-yl)-1,2,4-triazolo[1,5-*a*]quinoxaline-2-carboxylic acid (**9b**) and its corresponding ester (**9a**) are the most potent and selective AMPA receptor antagonists among the TQX series.

This result will be taken into consideration for the future design of novel potent and selective AMPA receptor antagonists.

Experimental Section

Chemistry. Silica gel plates (Merck F254) and silica gel 60 (Merck; 70−230 mesh) were used for analytical and column chromatography, respectively. All melting points were determined on a Gallenkamp melting point apparatus. Microanalyses were performed with a Perkin-Elmer 260 elemental analyzer for C, H, N, and the results were within ±0.4% of the theoretical values, except where stated otherwise (Table 4). The IR spectra were recorded with a Perkin-Elmer 1420 spectrometer in Nujol mulls and are expressed in cm^{-1}. The ^{1}H NMR spectra were obtained with a Varian Gemini 200 instrument at 200 MHz. The chemical shifts are reported in δ (ppm) and are relative to the central peak of the solvent. All the exchangeable protons were confirmed by addition of D_2O.

The physical and analytical data of the newly synthesized compounds are shown in Table 4.

Ethyl 4,5-Dihydro-8-nitro-4-oxo-7-trifluoromethyl-1,2,4-triazolo[1,5-*a*]quinoxaline-2-carboxylate (*10a*). A solution of ethyl 4,5-dihydro-7-trifluoromethyl-4-oxo-1,2,4-triazolo[1,5-*a*]quinoxaline-2-carboxylate[33] (6.13 mmol) in HNO_3 (90%, 20 mL) was heated at 40 °C for 1 h. The reaction mixture was poured onto ice (40 g) and the resulting solid was collected and washed with water: ^{1}H NMR (DMSO-d_6) 1.37 (t, 3H, CH_3, J = 6.96 Hz), 4.45 (q, 2H, CH_2, J = 6.96 Hz), 7.94 (s, 1H, ar), 8.87 (s, 1H, ar), 13.0 (br s, 1H, NH); IR 3190, 3085, 1720.

Ethyl 8-Amino-4,5-dihydro-4-oxo-7-trifluoromethyl-1,2,4-triazolo[1,5-*a*]quinoxaline-2-carboxylate (*11a*). Iron powder (3.0 g) was added to a solution of **10a** (3.0 mmol) in glacial acetic acid (18 mL). The mixture was heated at reflux for 1 h. Evaporation at reduced pressure of the solvent yielded a residue which was dried and extracted in Soxhlet with acetone (250 mL). Most of the solvent was evaporated to yield a solid which was collected by filtration: ^{1}H NMR (DMSO-d_6) 1.37 (t, 3H, CH_3, J = 6.96 Hz), 4.42 (q, 2H, CH_2, J = 6.96 Hz), 5.5 (br s, 2H, NH_2), 7.47 (s, 1H, ar), 7.64 (s, 1H, ar), 12.2 (br s 1H, NH); IR 3385, 1740, 1685.

Ethyl 4,5-Dihydro-4-oxo-8-(1,2,4-triazol-4-yl)-7-trifluoromethyl-1,2,4-triazolo[1,5-*a*]quinoxaline-2-carboxylate (*1a*). Diformylhydrazine (4.41 mmol) and then, drop by drop, trimethylsilyl chloride (22.6 mmol) and triethylamine (10.3 mmol) were added to a suspension of **11a** (1.47 mmol) in anhydrous pyridine (7 mL). The mixture was heated at 100 °C for an overall time of 72 h. The reaction was monitored by TLC [eluting system $CHCl_3$/MeOH (90:10)]. After 46 h, diformylhydrazine (1.24 mmol), trimethylsilyl chloride (6.1 mmol), and triethylamine (2.73 mmol) were added. Another addition of trimethylsilyl chloride (11.03 mmol) and triethylamine (5.02 mmol) was done after 56 h. Evaporation at reduced pressure of the solvent yielded a solid which was treated with water (50 mL), collected, and purified by silica gel column chromatography [eluting system $CHCl_3$/MeOH (90:10)]: ^{1}H NMR (DMSO-d_6) 1.35 (t, 3H, CH_3, J = 6.96 Hz), 4.43 (q, 2H, CH_2, J = 6.96 Hz), 7.93 (s, 1H, ar), 8.54 (s, 1H, ar), 8.83 (s, 2H, triazole H-2 and H-5),12.8 (br s, 1H, NH); IR 3140, 1735, 1715.

Table 4. Physical and Analytical Data of the Newly Synthesized Compounds

compd	mp (°C)	solvent[a]	yield (%)	C, H, N
1a	>300	A	64	$C_{15}H_{10}F_3N_7O_3$
1b	>300	B	95	$C_{13}H_6F_3N_7O_3$
2a	289 dec	C	67	$C_{18}H_{12}F_3N_5O_4$
2b	>300	B	72	$C_{16}H_8F_3N_5O_4$
3a	272−273	C	78	$C_{17}H_{12}F_3N_5O_3$
3b	>300	B	81	$C_{15}H_8F_3N_5O_3$
4a	262 dec	D	8	$C_{18}H_{12}F_3N_5O_5$
4b	250 dec	E	96	$C_{16}H_8F_3N_5O_5$
5a	>300	D	35	$C_{18}H_{10}F_3N_5O_6$
6a	266 dec	A	60	$C_{16}H_{11}F_3N_6O_3$
6b	>300	F	80	$C_{14}H_7F_3N_6O_3$
7a	>300	C	88	$C_{17}H_{12}N_6O_6$
7b	>300	C	95	$C_{15}H_8N_6O_6$
8a	276 dec	C	70	$C_{16}H_{12}N_6O_5$
8b	>300	B	90	$C_{14}H_8N_6O_5$
9a	237 dec	D	46	$C_{17}H_{12}N_6O_7$
9b	270 dec	B	90	$C_{15}H_8N_6O_7$
10a	292-293	A	92	$C_{13}H_8F_3N_5O_5$
10b	245 dec	A	87	$C_{11}H_4F_3N_5O_5$
11a	>300	A	70	$C_{13}H_{10}F_3N_5O_3$
11b	>300	F	44	$C_{11}H_6F_3N_5O_3$
12a	>300	C	75	$C_{14}H_{12}N_6O_6$
12b	>300	A	92	$C_{12}H_8N_6O_6$
13a	297 dec	A	56	$C_{12}H_{10}N_6O_5$
13b	>300	G	78	$C_{10}H_6N_6O_5$
14[b]	113−114	H	98	$C_7H_4ClF_3N_2O_2$
15[b]	223−224	H	60	$C_{10}H_7F_3N_4O_2$
16	145−147	H	40	$C_{14}H_{11}ClF_3N_5O_4$
17	224 dec	E	92	$C_{14}H_{13}F_3N_6O_4$
18	172−174	I	35	$C_{18}H_{15}F_3N_6O_6$
19	>300	A	89	$C_{14}H_{13}N_5O_4$

[a] Recrystallization solvents: A = ethanol; B = the title compound was dissolved in the minimal amount of 1 N NaOH, the insoluble material was filtered off, and the resulting clear solution acidified with 1 N HCl. The resulting solid was collected, treated with boiling ethanol, collected again, and washed with fresh ethanol; C = glacial acetic acid; D = purification of the title compound was performed by silica gel column cromatography as reported in the experimental; E = ethanol/diethyl ether; F = the title compound was treated as in B, but after dissolution in 1 N NaOH, the acid was precipitated by addition of glacial acetic acid; G = ethanol/water; H = ethyl acetate; I = ethyl acetate/diethyl ether. [b] Reference 38.

Ethyl 4,5-Dihydro-4-oxo-8-(3-formylpyrrol-1-yl)-7-trifluoromethyl-1,2,4-triazolo[1,5-*a*]quinoxaline-2-carboxylate (*2a*). A solution of 2,5-dimethoxytetrahydrofuran-3-carboxaldehyde (3.08 mmol) in glacial acetic acid (10 mL) was dropwise added to a suspension of **11a** (2.05 mmol) in glacial acetic acid (23 mL). The reaction mixture was heated at 90 °C for 10 min. After cooling, the mixture was diluted with water (200 mL) to yield a solid which was collected and washed with water. A second crop of **2a** was obtained by extracting the mother liquor with ethyl acetate (50 mL × 3). Evaporation of the dried (Na_2SO_4) organic layers afforded a solid which was purified by crystallization: ^{1}H NMR (DMSO-d_6) 1.35 (t, 3H, CH_3, J = 6.96 Hz), 4.43 (q, 2H, CH_2, J = 6.96 Hz), 6.68 (d, 1H, pyrrole H-4, J = 1,47 Hz), 7.17 (d, 1H, pyrrole H-5, J = 1.47 Hz), 7.93 (s, 2H, ar + pyrrole H-2), 8.26 (s, 1H, ar), 9.79 (s, 1H, CHO), 12.8 (br s, 1H, NH); IR 3100, 1755, 1720, 1640.

Ethyl 4,5-Dihydro-4-oxo-8-(pyrrol-1-yl)-7-trifluoromethyl-1,2,4-triazolo[1,5-*a*]quinoxaline-2-carboxylate (*3a*). A solution of 2,5-diethoxytetrahydrofuran (4.38 mmol) in glacial acetic acid (9 mL) was added dropwise to a hot (90 °C) suspension of **11a** (1.46 mmol) in glacial acetic acid (18 mL). The reaction mixture was kept at 90 °C for 5 min. Dilution with water (40 mL) afforded a solid which was collected and

washed with water: ^{1}H NMR (DMSO-d_6) 1.35 (t, 3H, CH_3, J = 6.96 Hz), 4.43 (q, 2H, CH_2, J = 6.96 Hz), 6.29 (t, 2H, pyrrole H-3 and H-4, J = 2.2 Hz), 7.01 (t, 2H, pyrrole H-2 and H-5, J = 2.2 Hz), 7.90 (s, 1H, ar), 8.02 (s, 1H, ar), 12.79 (br s, 1H, NH); IR 3270, 3070, 1740, 1710.

Ethyl 4,5-Dihydro-4-oxo-8-(3-carboxypyrrol-1-yl)-7-trifluoromethyl-1,2,4-triazolo[1,5-*a*]quinoxaline-2-carboxylate (*4a*) and Ethyl 4,5-Dihydro-4-oxo-8-(3-formyl-2,5-dioxopyrrol-1-yl)-7-trifluoromethyl-1,2,4-triazolo[1,5-*a*]quinoxaline-2-carboxylate (*5a*). An excess of solid potassium permanganate (3.0 mmol) was added portionwise to a cooled (0 °C) suspension of **2a** (1.19 mmol) in a 1:1 acetone/water mixture (10 mL). The reaction mixture was stirred at 0 °C for 4 h and then was diluted with ice/water (30 g); the excess of potassium permanganate was quenched with a 40% solution of sodium bisulfite and the resulting white solid was collected, washed with water, and purified by a silica gel column chromatography (eluting system $CHCl_3$/MeOH/AcOH 9:0.5:0.5). Evaporation of the first eluates gives **5a** (35% yield), while the second crops of eluates afforded the desired compound **4a** (7.2% yield). Compound **4a** displays the following spectral data: ^{1}HNMR (DMSO-d_6) 1.33 (t, 3H, CH_3, J = 6.96 Hz), 4.41 (q, 2H, CH_2, J = 6.96 Hz), 6.58 (d, 1H, pyrrole H-4, J = 1.47 Hz), 7.04 (d, 1H, pyrrole H-5, J = 1.47 Hz), 7.56 (s, 1H, pyrrole H-2), 7.88 (s, 1H, ar), 8.17 (s, 1H, ar), 12.1 (br s, 1H, COOH), 12.8 (br s, 1H, NH); IR 3560, 3415, 1735, 1695, 1670. Compound **5a** shows the following spectral data: ^{1}H NMR (DMSO-d_6) 1.36 (t, 3H, CH_3, J = 6.96 Hz), 4.43 (q, 2H, CH_2, J = 6.96 Hz), 7.76 (s, 1H, ar), 8.40 (s, 1H, ar), 8.71 (s, 1H, pyrrole H-4), 10.06 (s, 1H, CHO), 12.6 (br s, 1H, NH); IR 3240, 1740, 1720, 1655.

5-Chloro-2-nitro-4-trifluoromethylaniline (*14*).[38] The title compound was synthesized following the procedure reported in ref 38 with some modification. A mixture of the commercially available 2,4-dichloro-5-nitrobenzotrifluoride (7.7 mmol) in 2-methoxyethanol (6 mL) saturated with ammonia was heated in a sealed tube at 100 °C for 16 h. The reaction mixture was diluted with water (100 mL) and a solid precipitated which was collected and washed with water. ^{1}HNMR (DMSO-d_6) 7.25 (s, 1H, ar), 8.05 (s, 2H, NH_2), 8.27 (s, 1H, ar); IR 3510, 3400, 1640.

5-(Imidazol-1-yl)-2-nitro-4-trifluoromethylaniline (*15*).[38] 5-Chloro-2-nitro-4-trifluoromethylaniline **14** (4.16 mmol) was added to a solution of imidazole (16.0 mmol) and KOH (6.23 mmol) in anhydrous DMF (10 mL). The reaction mixture was heated at 100°C for 1.5 h. The resulting dark red solution was diluted with water (100 mL) and the solid which precipitated was extracted with ethyl acetate (50 mL × 3). The dried (Na_2SO_4) organic layers were decolored with animal carbon and evaporated to a small volume. The solid which crystallized was collected and washed with a little of diethyl ether. ^{1}H NMR (DMSO-d_6) 7.07−7.09 (m, 2H, ar + imidazole H-4), 7.41 (d, 1H, imidazole H-5, J = 1.22 Hz), 7.86 (s, 1H, imidazole H-2), 8.12 (s, 2H, NH_2), 8.37 (s, 1H, ar); IR 3460, 3280, 1650.

Ethyl N^1-[5-(Imidazol-1-yl)-2-nitro-4-trifluoromethylphenyl]hydrazono-N^2-chloroacetate (*16*). Concentrated H_2SO_4 (5.9 mL) was added to a suspension of **15** (3.67 mmol) in water (6 mL). A solution of $NaNO_2$ (5%, 5.6 mL) was added dropwise to the cooled (−5 °C) mixture, during an overall time of 45 min. Cold (0 °C) methanol (6 mL) and an equimolar amount of ethyl 2-chloro-3-oxobutanoate were added to the red solution, which was kept at 0 °C for 10 min and then at room temperature for 4 h. The yellow solution was diluted with water (80 mL) and extracted with chloroform (60 mL × 4). Evaporation of the dried (Na_2SO_4) organic layers yielded a solid which was treated with ethyl acetate and collected: ^{1}H NMR (DMSO-d_6) 1.26 (t, 3H, CH_3, J = 7.0 Hz), 4.33 (q, 2H, CH_2, J = 7.0 Hz), 7.18 (d, 1H, imidazole H-4, J = 1.22 Hz), 7.55 (d, 1H, imidazole H-5, J = 1.22 Hz), 7.70 (s, 1H, ar), 8.01 (s, 1H, imidazole H-2), 8.59 (s, 1H, ar), 11,20 (br s, 1H, NH); IR 3265, 1730, 1630.

Ethyl N^1-[5-(Imidazol-1-yl)-2-nitro-4-trifluoromethylphenyl]-N^2-oxamidrazonate (*17*). Ammonia was bubbled until saturation into a stirred solution of **16** (0.98 mmol) in anhydrous dioxane (8 mL). The mixture was stirred at room temperature for 1 h and then was diluted with water (30 mL) to yield an orange solid, which was collected and washed with water. ^{1}H NMR (DMSO-d_6) 1.22 (t, 3H, CH_3, J = 6.8 Hz), 4.24 (q, 2H, CH_2, J = 6.8 Hz), 7.11−7.13 (m, 3H, NH_2 + imidazole H-4), 7.47 (d, 1H, imidazole H-5, J = 1.22 Hz), 7.62 (s, 1H, ar), 7.92 (s, 1H, imidazole H-2), 8.46 (s, 1H, ar), 10.26 (br s, 1H, NH); IR 3420, 3300, 1710.

Diethyl 1-[5-(Imidazol-1-yl)-2-nitro-4-trifluoromethylphenyl]-1,2,4-triazolo]-3,5-dicarboxylate (*18*). Ethyloxalyl chloride (1.71 mmol) was added to a suspension of the amidrazone **17** (0.85 mmol) in anhydrous toluene (10 mL). The reaction mixture was heated at reflux for 3 h. Upon cooling, a solid precipitates which was collected and washed with a little diethyl ether: ^{1}HNMR (DMSO-d_6) 1.20 (t, 3H, CH_3, J = 7.0 Hz), 1.32 (t, 3H, CH_3, J = 7.0 Hz), 4.29 (q, 2H, CH_2, J = 7.0 Hz), 4.39 (q, 2H, CH_2, J = 7.0 Hz) 7.16 (s, 1H, imidazole H-4), 7.56 (s, 1H, imidazole H-5), 8.0 (s, 1H, imidazole H-2), 8.39 (s, 1H, ar), 8.84 (s, 1H, ar); IR 1740.

Ethyl 4,5-Dihydro-8-(imidazol-1-yl)-4-oxo-7-trifluoromethyl-1,2,4-triazolo[1,5-*a*]quinoxaline-2-carboxylate (*6a*). Iron powder (0.75 g) was added to a solution of **18** (0.75 mmol) in glacial acetic acid (6 mL). The mixture was heated at 90°C for 10 min. Evaporation at reduced pressure of the solvent yielded a residue which was treated with water (25 mL). The solid was collected by filtration and extracted in Soxlhet with acetone (250 mL). Evaporation of the solvent yielded a solid which was worked up with diethyl ether (10 mL) and collected. ^{1}H NMR (DMSO-d_6) 1.35 (t, 3H, CH_3, J = 6.96 Hz), 4.43 (q, 2H, CH_2, J = 6.96 Hz), 7.12 (s, 1H, imidazole H-4), 7.46 (s, 1H, imidazole H-5), 7.89 (s, 1H, imidazole H-2), 7.92 (s, 1H, ar), 8.22 (s, 1H, ar), 12.80 (br s, 1H, NH); IR 1750, 1710.

Ethyl 8-Acetylamino-4,5-dihydro-4-oxo-1,2,4-triazolo-[1,5-*a*]quinoxaline-2-carboxylate (*19*). Acetic anhydride (3.07 mmol) was added to a suspension of the ethyl 8-amino-4,5-dihydro-4-oxo-1,2,4-triazolo[1,5-*a*]quinoxaline-2-carboxylate[36] (2.56 mmol) in glacial acetic acid (18 mL). The reaction mixture was heated at reflux for 20 min. After cooling, the resulting solution was diluted with water (170 mL), affording a suspension which was stirred at room temperature for 2 h. The yellow solid was collected and washed with water: ^{1}H NMR (DMSO-d_6) 1.38 (t, 3H, CH_3, J = 6.96 Hz), 2.09 (s, 3H, CH_3), 4.44 (q, 2H, CH_2, J = 6.96 Hz), 7.44 (d, 1H, ar, J = 8.8 Hz), 7.58 (dd, 1H, ar, J = 8.8, 2.2 Hz), 8.64 (d, 1H, ar, J = 2.2 Hz), 10.3 (br s 1H, NH), 12.4 (br s 1H, NH); IR 3370, 1730, 1670.

Ethyl 8-Acetylamino-4,5-dihydro-7-nitro-4-oxo-1,2,4-triazolo[1,5-*a*]quinoxaline-2-carboxylate (*12a*). Compound **19** (2.22 mmol) was added portionwise to cold (−15 °C) HNO_3 (90%, 15 mL). The reaction mixture was stirred at −15 °C for 6 h and then was quenched with ice (70 g). The suspension was kept at room temperature for one night and the resulting solid was collected and washed with water: ^{1}H NMR (DMSO-d_6) 1.37 (t, 3H, CH_3, J = 6.96 Hz), 2.11 (s, 3H, CH_3), 4.44 (q, 2H, CH_2, J = 6.96 Hz), 7.80 (s, 1H, ar), 8.42 (s, 1H, ar), 10.4 (br s 1H, NH), 12.6 (br s 1H, NH); IR 3410, 1740, 1700, 1615.

Ethyl 8-Amino-4,5-dihydro-7-nitro-4-oxo-1,2,4-triazolo-[1,5-*a*]quinoxaline-2-carboxylate (*13a*). HCl (6 N, 5 mL) was added to a hot (90 °C) solution of **12a** (0.69 mmol) in EtOH (5 mL). The reaction mixture was heated at reflux for 1 h and then diluted with water (20 mL). The resulting solid was filtered, resuspended in water (10 mL), and basified with saturated $NaHCO_3$ solution. The suspension was kept at room temperature with stirring for 1 h. The orange solid was collected and washed with water ^{1}H NMR (DMSO-d_6) 1.36 (t, 3H, CH_3, J = 6.96 Hz), 4.43 (q, 2H, CH_2, J = 6.96 Hz), 7.5 (br s, 2H, NH_2), 7.79 (s, 1H, ar), 8.06 (s, 1H, ar), 12.2 (br s 1H, NH); IR 3470, 3210, 1755, 1725.

Ethyl 4,5-Dihydro-7-nitro-4-oxo-8-(3-formylpyrrol-1-yl)-1,2,4-triazolo[1,5-*a*]quinoxaline-2-carboxylate (*7a*). The title compound was prepared by reacting **13a** (2.05 mmol) with 2,5-dimethoxy-3-tetrahydrofurancarboxaldehyde (3.08 mmol) as described above for the preparation of **2a**: ^{1}H NMR (DMSO-

d_6) 1.35 (t, 3H, CH_3, J = 6.96 Hz), 4.43 (q, 2H, CH_2, J = 6.96 Hz), 6.67 (d, 1H, pyrrole, H-4, J = 1.47 Hz), 7.24 (d, 1H, pyrrole H-5, J = 1.47 Hz), 8.04 (s, 1H, pyrrole H-2), 8.18 (s, 1H, ar), 8.32 (s, 1H, ar), 9,77 (s, 1H, CHO), 12.9 (br s, 1H, NH); IR 3140, 1745, 1715, 1655.

Ethyl 4,5-Dihydro-7-nitro-4-oxo-8-(pyrrol-1-yl)-1,2,4-triazolo[1,5-*a*]quinoxaline-2-carboxylate (*8a*). The title compound was prepared by reacting **13a** (1.25 mmol) with 2,5-diethoxytetrahydrofuran (3.76 mmol) as described above for the preparation of **3a**: ^{1}H NMR (DMSO-d_6) 1.35 (t, 3H, CH_3, J = 6.98 Hz), 4.43 (q, 2H, CH_2, J = 6.98 Hz), 6.29 (t, 2H, pyrrole H-3 and H-4, J = 2.19 Hz), 7.06 (t, 2H, pyrrole H-2 and H-5, J = 2.19 Hz), 8.06 (s, 1H, ar), 8.10 (s, 1H, ar), 12.8 (br s, 1H, NH); IR 3160, 3070, 1715.

Ethyl 4,5-Dihydro-7-nitro-4-oxo-8-(3-carboxypyrrol-1-yl)-1,2,4-triazolo[1,5-*a*]quinoxaline-2-carboxylate (*9a*). An excess of solid potassium permanganate (1.2 mmol) was portionwise added to a cooled (0−5 °C) suspension of **7a** (0.48 mmol) in a 1:1 acetone/water mixture (10 mL). Small additions were done in sequence at the disappearance of the characteristic violet color of the oxidizing agent, in a total time of 10 h. After stirring at room temperature for one night, the reaction mixture was then diluted with ice−water (20 g) and the excess of potassium permanganate was quenched with a 40% solution of sodium bisulfite. The resulting solution was extracted with ethyl acetate (50 mL × 3), and then evaporation of the dried (Na_2SO_4) organic layers afforded a solid which was collected and purified by silica gel column chromatography [eluting system $CHCl_3$/EtOH (9:1)]: ^{1}H NMR (DMSO-d_6) 1.34 (t, 3H, CH_3, J = 6.96 Hz), 4.41 (q, 2H, CH_2, J = 6.96 Hz), 6.58 (d, 1H, pyrrole H-4, J = 1.47 Hz), 7.12 (d, 1H, pyrrole H-5, J = 1.47 Hz), 7.68 (s, 1H, pyrrole H-2), 8.13 (s, 1H, ar), 8.24 (s, 1H, ar), 12.1 (br s, 1H, COOH), 12.9 (br s, 1H, NH); IR 1740, 1695.

4,5-Dihydro-4-oxo-8-(1,2,4-triazol-4-yl)-7-trifluoromethyl-1,2,4-triazolo[1,5-*a*]quinoxaline-2-carboxylic Acid (*1b*). A solution of NaOH (3%, 11 mL) was added to a suspension of **1a** (0.46 mmol) in EtOH (11 mL). The mixture was stirred at room temperature for 1.5 h. The solid was collected by filtration and dissolved in the minimal amount of water; acidification of the cold (5 °C) and clear solution by 6 N HCl yielded a solid that, after standing 3 h in a ice bath, was collected and washed with water: ^{1}H NMR (DMSO-d_6) 7.92 (s, 1H, ar), 8.47 (s, 1H, ar), 8.83 (s, 2H, triazole H-2 and H-5), 12.8 (br s, 1H, NH); IR 3520, 3360, 3140, 1725, 1685.

4,5-Dihydro-4-oxo-8-(3-formylpyrrol-1-yl)-7-trifluoromethyl-1,2,4-triazolo[1,5-*a*]quinoxaline-2-carboxylic Acid (*2b*). A solution of NaOH (3%, 8 mL) was added to a suspension of **2a** (0.29 mmol) in EtOH (9 mL). The mixture was stirred at room temperature for 15 min and then diluted with water (20 mL). After cooling (0−5°C), the resulting suspension was acidified with 6 N HCl and kept in an ice bath for one night. The solid was collected by filtration and washed with water: ^{1}H NMR (DMSO-d_6) 6.67 (d, 1H, pyrrole H-4, J = 1,47 Hz), 7.16 (d, 1H, pyrrole H-5, J = 1.47 Hz), 7.93 (s, 2H, ar + pyrrole H-2), 8.22 (s, 1H, ar), 9.78 (s, 1H, CHO), 12.82 (s, 1H, NH); IR 1720, 1675, 1650.

4,5-Dihydro-4-oxo-8-(pyrrol-1-yl)-7-trifluoromethyl-1,2,4-triazolo[1,5-*a*]quinoxaline-2-carboxylic Acid (*3b*). A solution of NaOH (3%, 13.5 mL) was added to a suspension of **3a** (0.51 mmol) in EtOH (14 mL). The reaction mixture was stirred at room temperature for 5 min and then, after cooling (0−5 °C), was acidified with 6 N HCl. The resulting suspension was stirred in an ice bath for 2 h and then the solid was collected and washed with water: ^{1}H NMR (DMSO-d_6) 6.29 (t, 2H, pyrrole H-3 and H-4, J = 2.2 Hz), 7.00 (t, 2H, pyrrole H-2 and H-5, J = 2.2 Hz), 7.92 (s, 1H, ar), 7.99 (s, 1H, ar), 12.81 (s, 1H, NH); IR 3195, 1730, 1715.

4,5-Dihydro-4-oxo-8-(3-carboxypyrrol-1-yl)-7-trifluoromethyl-1,2,4-triazolo[1,5-*a*]quinoxaline-2-carboxylic Acid (*4b*). A solution of NaOH (0.8 N, 5.8 mL) was added to a suspension of **4a** (0.23 mmol) in EtOH (5 mL). The reaction mixture was stirred at room temperature for 1.5 h and then, after cooling (0−5 °C), was acidified with 6 N HCl. The resulting suspension was extracted with ethyl acetate (15 mL × 2). Evaporation of the dried (Na_2SO_4) organic layers yielded a solid which was worked up with a little diethyl ether and collected by filtration: ^{1}H NMR (DMSO-d_6) 6.59 (d, 1H, pyrrole H-4, J = 1.47 Hz), 7.07 (d, 1H, pyrrole H-5, J = 1.47 Hz), 7.57 (s, 1H, pyrrole H-2), 7.90 (s, 1H, ar), 8.16 (s, 1H, ar), 12.8 (br s, 1H, NH); IR 3600−3000, 1700, 1670.

4,5-Dihydro-8-(imidazol-1-yl)-4-oxo-7-trifluoromethyl-1,2,4-triazolo[1,5-*a*]quinoxaline-2-carboxylic Acid (*6b*). A solution of NaOH (1 N, 2 mL) was added to a suspension of **6a** (0.1 mmol) in EtOH (2.5 mL). The reaction mixture was stirred at room temperature for 1 h and then was acidified with glacial acetic acid. The resulting solid was collected and washed with water: ^{1}H NMR (DMSO-d_6) 7.11 (s, 1H, imidazole H-4), 7.45 (s, 1H, imidazole H-5), 7.88 (s, 1H, imidazole H-2), 7.92 (s, 1H, ar), 8.17 (s, 1H, ar), 12.8 (br s, 1H, NH); IR 3600−3000, 1695, 1605.

4,5-Dihydro-7-nitro-4-oxo-8-(3-formylpyrrol-1-yl)-1,2,4-triazolo[1,5-*a*]quinoxaline-2-carboxylic Acid (*7b*). A solution of NaOH (3%, 12 mL) was added to a suspention of **7a** (0.5 mmol) in EtOH (13 mL). The reaction mixture was stirred at room temperature for 1 h, diluted with water (10 mL), and acidified with 6 N HCl. The resulting solid was collected by filtration and washed with water. A second crop of **7b** was obtained by extracting the acidic mother liquor with ethyl acetate (30 mL × 3); evaporation of the dried (Na_2SO_4) organic layers yielded a solid which was worked up with a small amount of diethyl ether and filtered. ^{1}H NMR (DMSO-d_6) 6.67 (d, 1H, pyrrole, H-4, J = 1.47 Hz), 7.25 (d, 1H, pyrrole H-5, J = 1.47 Hz), 8.04 (s, 1H, pyrrole H-2), 8,18 (s, 1H, ar), 8.29 (s, 1H, ar), 9,77 (s, 1H, CHO), 12.9 (br s, 1H, NH); IR 3140, 1735, 1675.

4,5-Dihydro-7-nitro-4-oxo-8-(pyrrol-1-yl)-1,2,4-triazolo-[1,5-*a*]quinoxaline-2-carboxylic Acid (*8b*). A solution of NaOH (3%, 10 mL) was added to a suspension of **8a** (0.41 mmol) in EtOH (10 mL). The reaction mixture was stirred at room temperature for 5 min and then was acidified with 6 N HCl. After stirring at room temperature for 3 h, the suspension was filtered under vacuum and the resulting solid was washed with water: ^{1}H NMR (DMSO-d_6) 6.29 (t, 2H, pyrrole H-3 and H-4, J = 2.2 Hz), 7.05 (t, 2H, pyrrole H-2 and H-5, J = 2.2 Hz), 8.05 (s, 1H, ar), 8.09 (s, 1H, ar), 12.8 (br s, 1H, NH); IR 3130, 1730.

4,5-Dihydro-7-nitro-4-oxo-8-(3-carboxypyrrol-1-yl)-1,2,4-triazolo[1,5-*a*]quinoxaline-2-carboxylic Acid (*9b*). A solution of NaOH (0.8 N, 2.5 mL) was added to a suspension of **9b** (0.097 mmol) in EtOH (2.5 mL). The reaction mixture was stirred at room temperature for 1 h and then was diluted with water (10 mL). The resulting solution was decolored with activated carbon, acidified with 6 N HCl, and then extracted with ethyl acetate (15 mL × 3). Evaporation of the dried (Na_2SO_4) organic layers yielded a yellow solid which was worked up with a little diethyl ether and collected by filtration: ^{1}HNMR (DMSO-d_6) 6.61 (d, 1H, pyrrole H-4, J = 1.47 Hz), 7.14 (d, 1H, pyrrole H-5, J = 1.47 Hz), 7.71 (s, 1H, pyrrole H-2), 8.16 (s, 1H, ar), 8.25 (s, 1H, ar), 12.2 (br s, 1H, COOH), 12.88 (s, 1H, NH); IR 1730.

4,5-Dihydro-8-nitro-4-oxo-7-trifluoromethyl-1,2,4-triazolo[1,5-*a*]quinoxaline-2-carboxylic Acid (*10b*). A solution of NaOH (3%, 10 mL) was added to a suspension of **10a** (0.4 mmol) in EtOH (11 mL). The reaction mixture was stirred at room temperature for 3 h. The solid was collected and dissolved in the minimum amount of water. The resulting solution was acidified with 6 N HCl and then kept at room temperature for 30 min. The suspension was filtered under vacuum and the solid was washed with water: ^{1}H NMR(DMSO-d_6) 7.93 (s, 1H, ar), 8.84 (s, 1H, ar), 13.0 (br s, 1H, NH); IR 1735.

8-Amino-4,5-dihydro-4-oxo-7-trifluoromethyl-1,2,4-triazolo[1,5-*a*]quinoxaline-2-carboxylic Acid (*11b*). A solution of NaOH (1 N, 4.5 mL) was added to a suspension of **11a** (0.73 mmol) in EtOH (3.7 mL). The reaction mixture was heated at 90°C for 1 h. After cooling, a solid precipitated which was collected and dissolved in a small amount of water (3 mL). Acidification with glacial acetic acid of the resulting solution yielded a solid which was collected and washed with water. A

second crop of **10b** was obtained after evaporation, at small volume, of the hydroalcolic alkaline mother liquor and acidification with glacial acetic acid. The resulting solid was collected and washed with water: ^{1}H NMR (DMSO-d_6) 5.87 (s, 2H, NH_2), 7.47 (s, 1H, ar), 7.60 (s, 1H, ar), 12.12 (s 1H, NH); IR 3600, 3380, 3260, 1685.

8-Acetylamino-4,5-dihydro-7-nitro-4-oxo-1,2,4-triazolo-[1,5-*a*]quinoxaline-2-carboxylic Acid (*12b*). A solution of NaOH (3%, 15 mL) was added to a suspension of **12a** (0.55 mmol) in EtOH (15 mL). The reaction mixture was stirred at room temperature for 30 min and then was acidified with glacial acetic acid. The resulting suspension was kept at room temperature for 30 min and then the solid was collected and washed with water: ^{1}H NMR (DMSO-d_6) 2.11 (s, 3H, CH_3), 7.91 (s, 1H, ar), 8.31 (s, 1H, ar), 10.36 (s, 1H, NH), 12.3 (br s 1H, NH); IR 3230, 1725, 1680.

8-Amino-4,5-dihydro-7-nitro-4-oxo-1,2,4-triazolo[1,5-*a*]quinoxaline-2-carboxylic Acid (*13b*). A solution of **13a** (0.22 mmol) in concentrated HCl (2 mL) was heated at reflux for 1 h. The resulting solid was collected and washed with water: ^{1}H NMR (DMSO-d_6) 7.4 (br s, 2H, NH_2), 7.77 (s, 1H, ar), 8.07 (s, 1H, ar), 12.21 (s, 1H, NH); IR 3480, 3370, 3280, 1700, 1650.

Pharmacology. Binding Assay. Rat cortical synaptic membrane preparation, [^{3}H]glycine, [^{3}H]AMPA, and [^{3}H]-(+)-MK-801 binding experiments were performed following the procedures described in refs 30, 45, and 35, respectively.

[^{3}H]Kainate Binding. High-affinity and low-affinity [^{3}H]-kainate binding assays were performed on rat cortical membranes according to the method of Johansen et al.[12] The standard high-affinity assay was performed at 2 nM [^{3}H]-kainate (NEN Life Science Products; Boston, MA; specific activity 58 Ci/mmol) in Tris-HCl buffer (50 mM, pH 7.4), whereas the low-affinity binding assay was performed at 20 nM [^{3}H]kainate in Tris-HCl buffer containing 20 mM calcium chloride. Binding was terminated by rapid filtration over glass fiber filters (Millipore APFC02500). Non-specific binding was determined in the presence of 1 mM glutamate.

Electrophysiological Assay. The cortical wedge preparation described by Mannaioni et al.[46] was used while the electrophysiological assays were performed following the procedures described in ref 37.

[^{3}H]NE Release from Synaptosomes. The following procedure was adapted from the method of Pittaluga et al.;[47] cyclothiazide was added to potentiate AMPA-induced [^{3}H]-norepinephrine release.[48] Briefly, crude synaptosomes prepared from rat hippocampi were resuspended in an oxygenated (95% O_2 and 5% CO_2) physiological standard medium having the following composition (mM): NaCl 125, KCl 3, $MgSO_4$ 1.2, $CaCl_2$ 1.2, NaH_2PO_4 1.0, $NaHCO_3$ 22, glucose 10; pH 7.2−7.4. Synaptosomes were then incubated at 37 °C for 15 min with 50 nM levo-[*ring*-2,5,6-^{3}H]norepinephrine (NEN Life Science Products; specific activity 56.4 Ci/mmol) in the presence of 1 μM 6-nitroquipazine (Sigma-RBI) to prevent [^{3}H]catecholamine uptake by serotoninergic nerve terminals. Identical aliquots of the labeled synaptosomal suspension were then introduced in parallel superfusion chambers maintained at 37 °C. Superfusion was carried out at a rate of 0.6 mL/min, using media aerated with 95% O_2–5% CO_2, for a total period of 58 min. After 46 min of superfusion to equilibrate the system, four consecutive 3 min fractions were collected. Cyclothiazide (Sigma-RBI) (50 μM) was added 6 min prior to collecting the first fraction. S-AMPA (Tocris) (50 μM) was introduced 1 min before completing the first fraction collection, while antagonists were added 8 min before AMPA, that is, together with cyclothiazide. The fractions were collected and the superfused synaptosomes counted for radioactivity.

The amount of radioactivity released into each superfusate fraction was expressed as a percentage of the total synaptosomal radioactivity at the start of the collected fraction. Drug effects were evaluated from the ratio between the [^{3}H]-norepinephrine release in the fraction corresponding to the maximal effect (third fraction) and that in the first fraction (basal efflux). This ratio was compared with the corresponding ratio obtained under control conditions (buffer plus 50 μM cyclothiazide only).

IC_{50} values for antagonists were determined from inhibition curves, based on four different drug concentrations, using a function fitting program (ALLFIT).

Sample Preparation and Result Calculation. A stock 1 mM solution of the test compound was prepared in 50% DMSO. Subsequent dilutions were accomplished in buffer. The IC_{50} values were calculated from three or four displacement curves based on four to six scalar concentrations of the test compound in triplicate using the ALLFIT computer program[49] and, in the case of tritiated glycine and AMPA binding, converted to K_i values by application of the Cheng−Prusoff equation.[50] Under our experimental conditions the dissociation constants (K_D) for [^{3}H]glycine (10 nM) and [^{3}H]-DL-AMPA (8 nM) were 75 ± 6 and 28 ± 3 nM, respectively.

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