Synthesis, Ionotropic Glutamate Receptor Binding Affinity, and Structure−Activity Relationships of a New Set of 4,5-Dihydro-8-heteroaryl-4-oxo-1,2,4-triazolo[1,5-*a*]quinoxaline-2-carboxylates Analogues of TQX-173

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A series of 4,5-dihydro-4-oxo-1,2,4-triazolo[1,5-*a*]quinoxaline-2-carboxylates analogues of TQX-173 (**1b**), bearing different nitrogen-containing heterocycles at position-8, were synthesized as AMPA receptor antagonists. All the reported compounds were also biologically evaluated for their binding at glycine/NMDA and KA receptors to better assess their selectivity toward the AMPA receptor. Structure−activity relationships (SAR) on these TQX derivatives have evidenced that the precise positioning of the nitrogen atoms and the specific electronic topography of the 8-heteroaromatic ring are both important for the anchoring to the AMPA receptor. In fact, it has been 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. Functional antagonism at both AMPA receptor and NMDA receptor-ion channel complex was evaluated by assessing the ability of some selected compounds to inhibit depolarization induced by 5 μM AMPA or NMDA in mouse cortical wedge preparations.

Introduction

It has been well-established that many neurological disorders are caused by excessive release of glutamate (Glu) from presynaptic terminals which overstimulate postsynaptic glutamate receptors (GluRs). GluRs mediate neurotransmission at the majority of fast excitatory synapses in the mammalian central nervous system. The postsynaptic activity of Glu is mediated by both ionotropic receptors (iGluRs), ligand-gated ion channels, and metabotropic receptors (mGluRs), coupled to G-proteins. Historically the iGluRs were broadly subdivided into *N*-methyl-D-aspartate (NMDA), (*R*,*S*)-2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)propionic acid (AMPA), and kainate (KA) receptors on the basis of their respective affinities for these exogenous amino acids.[1]

Competitive iGluR antagonists have been proposed as potentially useful neuroprotective agents for the prevention and treatment of several acute and chronic neurological disorders.[1−4] Moreover, an alternative approach to antagonize the overstimulation of postsynaptic GluRs by excessive endogenous Glu was represented by the use of noncompetitive NMDA antagonists acting at the strychnine-insensitive glycine site (Gly/NMDA) on the NMDA receptor.[5−7] In fact, a unique feature of the NMDA receptor is the presence of an allosteric modulatory site at which the amino acid glycine acts as a necessary coagonist in receptor activation.[8] Thus, blockage of the Gly/NMDA site by antagonists results in a complete inhibition of NMDA-mediated neurodegenerative effects.

There has been considerable interest in mixed AMPA and Gly/NMDA antagonists as well as selective AMPA antagonists that have demonstrated significant neuroprotective action in several models of cerebral ischemia and neuronal injury and have provided the basis for extensive research in this area.[2−4,9−10]

As part of a program aimed at finding novel competitive and noncompetitive iGluR antagonists[11−17], we previously studied a set of 4,5-dihydro-4-oxo-1,2,4-triazolo[1,5-*a*]quinoxaline-2-carboxylates (TQXs) that possessed combined Gly/NMDA and AMPA receptor affinity,[15] thus providing further evidence of the structural similarities and differences of the binding pockets of both receptor recognition sites.[2,18] On this basis we hypothesized that the TQX framework, suitably modified, could lead to selective AMPA receptor antagonists.

Thus, we have recently reported, in preliminary form,[16] the AMPA receptor selective antagonists 7-chloro-4,5-dihydro-4-oxo-1,2,4-triazolo[1,5-*a*]quinoxaline-2-carboxylates **1a**,**b** and **2a,b**, each bearing at position-8 a (1,2,4-triazol-4-yl) and a (imidazol-1-yl) moiety (Chart 1). Compounds **2a,b** were rationally designed as AMPA receptor antagonists.[3,19] The emerging novelty of the work[16] is the fact that introduction of the 8-(1,2,4-triazol-4-yl) substituent has led to a more potent and selective AMPA receptor antagonist (TQX-173, i.e., compound **1b**) than that bearing the claimed 8-(imidazol-1-yl) moiety (compound **2b**).

To shed more light on the SAR of these triazoloquinoxaline derivatives, we report, in this fully detailed version, the synthesis and binding affinity at AMPA,

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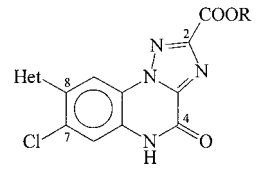
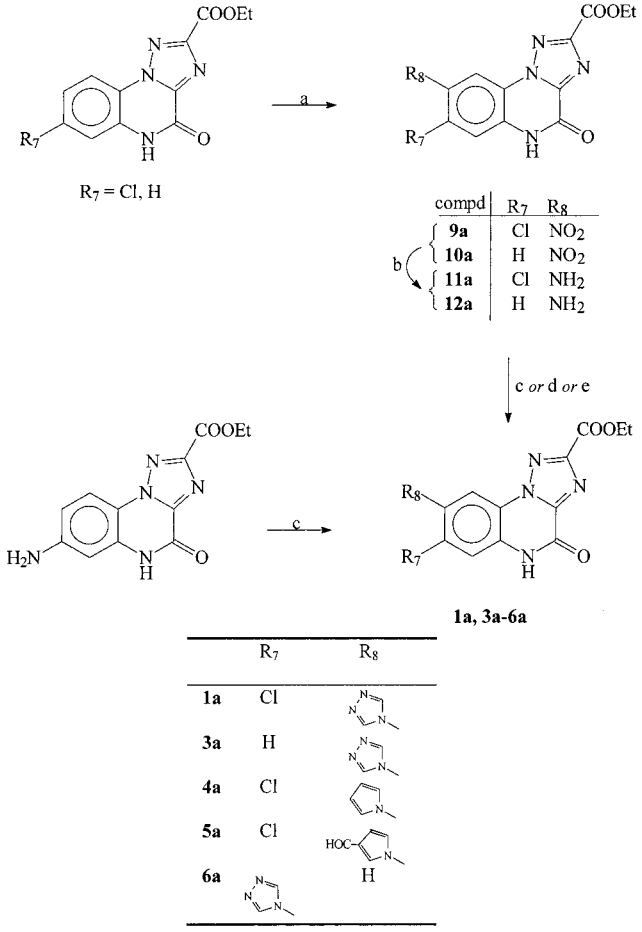
Chart 1



	R	Het
1a	Et	
1b (TQX-173)	H	
2a	Et	
2b	H	

Scheme 1[a]



compd	R_7	R_8
9a	Cl	NO_2
10a	H	NO_2
11a	Cl	NH_2
12a	H	NH_2

	R_7	R_8
1a	Cl	
3a	H	
4a	Cl	
5a	Cl	
6a		

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

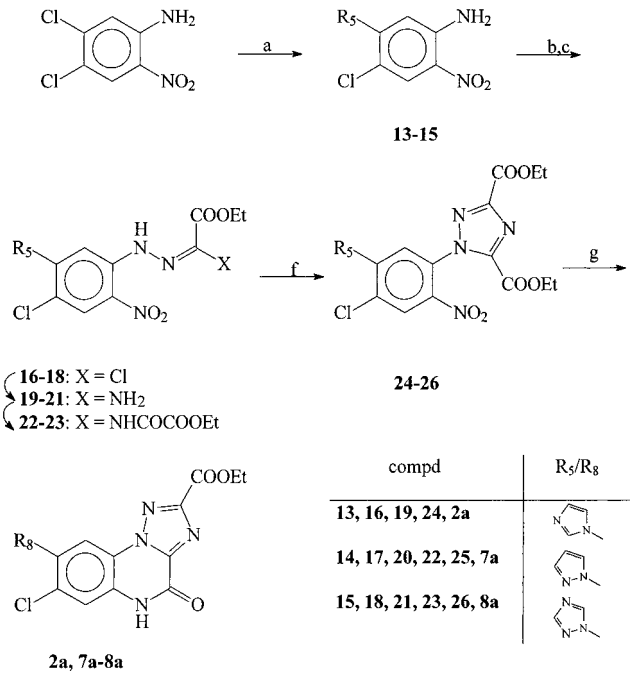
Gly/NMDA, and KA receptors of all the triazoloquinoxaline-2-carboxylates bearing different substituents at position-8.

Chemistry

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

The previously reported ethyl 7-chloro-4,5-dihydro-4-oxo-1,2,4-triazolo[1,5-*a*]quinoxaline-2-carboxylate[15] and its 7-unsubstituted analog[15] were regioselectively nitrated at position-8 to yield **9a**[15] and **10a**, respectively, which were reduced to their corresponding 8-amino derivatives **11a**[16] and **12a** (Scheme 1). By reacting **11a** and **12a** with diformylhydrazine, the 8-(1,2,4-triazol-4-yl) esters **1a**[16] and **3a** were prepared. The 8-(pyrrol-1-yl) ester **4a** and the 8-(3-formylpyrrol-1-yl) ester **5a** were obtained by reacting **11a** either with 2,5-diethoxytetrahydrofuran or 2,5-dimethoxy-3-tetrahydrofurancarboxaldehyde, respectively. The 7-(1,2,4-triazol-4-yl) ester **6a** was obtained from the reaction of ethyl 7-amino-4,5-dihydro-4-oxo-1,2,4-triazolo[1,5-*a*]quinoxaline-2-carboxylate[15] with diformylhydrazine (Scheme 1).

Scheme 2[a]



compd	R_5/R_8
13, 16, 19, 24, 2a	
14, 17, 20, 22, 25, 7a	
15, 18, 21, 23, 26, 8a	

[a] Reagents and conditions: (a) Het-H, KOH, DMF; (b) $NaNO_2$/conc H_2SO_4, $NaBF_4$; (c) $CH_3COCHClCOOEt$; (d) NH_3(g), dioxane.; (e) ClCOCOOEt, toluene; (f) conc H_2SO_4; (g) iron, AcOH.

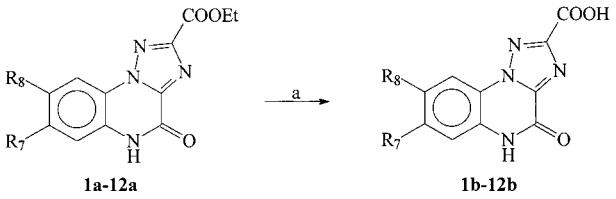
By reacting the commercially available 4,5-dichloro-2-nitroaniline with the suitable heterocycle, the corresponding 4-chloro-5-heteroaryl-2-nitroanilines **13**[16,20]−**15** were isolated (Scheme 2). The diazonium tetrafluoborates of **13**−**15** were reacted with ethyl 2-chloro-3-oxobutanoate to yield the N^2-chloroacetates **16**[16]−**18**, which were transformed with ammonia into their corresponding N^2-oxamidrazonates **19**[16]−**21**. Reaction of **20** and **21** with ethyloxalyl chloride yielded the N^3-ethoxalyl derivatives **22** and **23**, respectively. By reacting the N^2-oxamidrazonate **19** with ethyloxalyl chloride the diethyl 1-[4-chloro-5-(imidazol-1-yl)-2-nitrophenyl]-1,2,4-triazole-3,5-dicarboxylate **24**[16] was directly obtained. The diethyl triazole-3,5-dicaboxylates **25** and **26** were obtained by cyclization with concentrated H_2SO_4 of the N^3-ethoxalyl derivatives **22** and **23**, respectively. Reduction of the nitro group of **24**−**26** afforded the tricyclic esters **2a**[16],**7a**, and **8a**.

Finally, the hydrolysis of the esters **1a**−**12a** to their corresponding acids **1b**−**12b** is depicted in Scheme 3.

Results and Conclusions

The triazoloquinoxalines **1a,b**−**12a,b**, together with NBQX (2,3-dihydroxy-6-nitro-7-sulfamoylbenzo[*f*]qui-

Scheme 3[a]



compd	R_7	R_8
1a, 1b	Cl	
2a, 2b	Cl	
3a, 3b	H	
4a, 4b	Cl	
5a, 5b	Cl	
6a, 6b		H
7a, 7b	Cl	
8a, 8b	Cl	
9a, 9b	Cl	NO_2
10a, 10b	H	NO_2
11a, 11b	Cl	NH_2
12a, 12b	H	NH_2

[a] Reagents and conditions: (a) 0.8 N NaOH, EtOH, 6 N HCl or AcOH.

noxaline) and DCKA (5,7-dichlorokynurenic acid) as standard compounds, were tested for their ability to displace tritiated AMPA, glycine, and KA from their specific binding sites in rat cortical membranes. The binding results, shown in Table 1, indicate that introduction of different heteroaromatic rings at position-8, holding the 7-chloro substitution pattern constant, leads to some AMPA receptor antagonists belonging to the TQX series. In particular, the previously reported 7-chloro-4,5-dihydro-8-(1,2,4-triazol-4-yl)-4-oxo-1,2,4-triazolo[1,5-*a*]quinoxaline-2-carboxylates **1a,b**[16] are the most potent and selective AMPA receptor antagonists among the herein reported compounds. These data point out that the (1,2,4-triazol-4-yl) moiety at position-8 of these tricyclic derivatives could be an adequate substituent in distinguishing the AMPA receptor from the glycine/NMDA site.

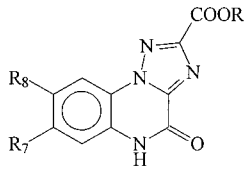
We previously reported that the presence of a free carboxylic group at position-2 on the TQX framework was not an essential feature for the anchoring to the AMPA receptor.[15] On the basis of the present study, this statement can be generally accepted, but it is necessary to go into greater depth. In fact, as with most of the previously reported combined glycine/NMDA and AMPA antagonists belonging to the TQX series,[15] the carboxylic acid derivatives **1b**, **2b**,[16] and **8b**, bearing a N^3-nitrogen-containing heterocycle at position-8, are only 2−5-fold more active at the AMPA receptor than their corresponding esters **1a**, **2a**,[16] and **8a**, respectively. On the contrary, the 2-carboxylic acid derivatives **4b** and **7b**, bearing a 8-heterocyclic ring lacking the N^3- nitrogen atom, are about 20-fold more active than their corresponding ester derivatives **4a** and **7a**. In fact, removal of the N^3-nitrogen from the imidazole ring at position-8 of compounds **2a,b** to give the 8-(pyrrol-1-yl) derivatives **4a,b** causes an 8-fold reduction in potency in the 2-carboxylic ester derivative (compare **4a** to **2a**) but does not affect the binding affinity of the 2-carboxylic acid derivative (compare **4b** to **2b**). The same applies to the 8-(pyrazol-1-yl) derivatives **7a,b** with respect to those bearing the 8-(1,2,4-triazol-1-yl) moiety **8a,b**. Thus, these results indicate that the presence of a free carboxylic group at position-2 is not an essential feature when a N^3-nitrogen-containing heterocycle is present at position-8.

These data led to the hypothesis that the N^3-nitrogen atom could act as a proton acceptor in a hydrogen bond with a receptor site, thus reinforcing the receptor−ligand interaction.[10,18] This hypothesis is confirmed by the binding affinity of compound **5a**, which is formally obtained by the introduction of a 3-formyl group on the 8-(pyrrol-1-yl) moiety of **4a**. In fact, **5a** shows a 10-fold increased AMPA binding affinity with respect to **4a** but is comparable to that of the 8-(imidazol-1-yl) derivative **2a**. Furthermore, the 2-carboxylic acid **5b** is only 3-fold more active at the AMPA receptor than the corresponding ester **5a**. These data indicate that the 3-formyl group could replace the N^3-nitrogen atom of the 8-heteroaryl ring acting as a proton acceptor group in a hypothetical hydrogen bond receptor−ligand interaction.

Furthermore, the AMPA binding results of the 7-chloro-8-heteroaryl-2-carboxylic acid derivatives **1b**, **2b**, **4b**, **5b**, **7b**, and **8b** indicate that the precise positioning of the nitrogen atoms and the specific electronic topography of the 8-heterocyclic rings are both important for potent but also selective AMPA receptor antagonists belonging to the TQX series. In fact, compound **4b**, which bears the pyrrole ring at position-8, is equipotent at both AMPA and Gly/NMDA receptors. Introduction of a N^3-nitrogen atom on the 8-(pyrrol-1-yl) moiety of **4b** gives the potent and AMPA selective 8-(imidazol-1-yl) derivative **2b.** On the contrary, introduction of a N^2-nitrogen atom on the pyrrole ring of **4b** yields the 8-(pyrazol-1-yl) derivative **7b**, which is as nonselective as the parent compound **4b**. These results are confirmed by the binding data of the compound **8b**, which bears both the N^2- and N^3 (i.e., the N^4 of the 8-(1,2,4-triazol-1-yl) moiety) nitrogen atoms on the heterocyclic ring. In fact, because of the beneficial effect of the N^3-nitrogen atom, compound **8b,** though bearing the N^2-nitrogen, is as selective as **2b**.

Replacement of the 8-nitro group of **9b**[15,16] with an amino one gives compound **11b**, which has an AMPA binding affinity comparable to that of **9b**. The same applies to the corresponding 2-carboxylic ester **11a** with respect to **9a**. The similar effect exerted by both the nitro and the amino groups on the AMPA binding affinity might be explained as follows. The 8-nitro substituent, due to its electron-withdrawing effect, could increase the NH lactame acidity, thus reinforcing the essential hydrogen-bond receptor−ligand interaction at this level.[2,10,18,19] On the other hand, the 8-amino group could act as a proton acceptor in a hydrogen-bond interaction with a receptor site.[2,10,18] The unquestionably different nature of the nitro and amino group might explain the reduced difference between the K_i values of **11a,b** relative to that of **9a,b**. In fact, while the 8-amino-

Table 1. Binding Activity at iGluRs of TQX Derivatives[a]



compd	R	R_7	R_8	[^{3}H]AMPA K_i (μM)[b] or I%[c]	[^{3}H]glycine K_i (μM)[b] or I%[c]	[^{3}H]KA IC_{50} (μM)[d] or I%[c]
1a[e]	Et	Cl		0.70 ± 0.13	15%	42%
1b (TQX-173)[e]	H	Cl		0.14 ± 0.02	33.5 ± 5.3	11.6 ± 1.3
2a[e]	Et	Cl		2.30 ± 0.13	39%	24%
2b[e]	H	Cl		0.98 ± 0.04	45%	16.8 ± 3.3
3a	Et	H		0%	0%	20%
3b	H	H		17.2 ± 3.5	0%	90 ± 11
4a	Et	Cl		18.4 ± 4.2	23 ± 5.7	7%
4b	H	Cl		0.86 ± 0.14	0.86 ± 0.09	36 ± 5
5a	Et	Cl		1.9 ± 0.09	19 ± 5.5	88 ± 9
5b	H	Cl		0.53 ± 0.04	28 ± 5.3	8.9 ± 0.6
6a	Et		H	10.1 ± 1	8%	35%
6b	H		H	4.3 ± 0.8	0%	44%
7a	Et	Cl		44%	7.1 ± 0.9	20%
7b	H	Cl		4.3 ± 0.8	5.3 ± 0.9	20 ± 0.8
8a	Et	Cl		17.2 ± 1.7	28%	29%
8b	H	Cl		3.7 ± 0.3	35%	48 ± 6
9a	Et	Cl	NO_2	47 ± 12	10.24 ± 2.1	35%
9b	H	Cl	NO_2	1.2 ± 0.08[f]	2.2 ± 0.3[f]	41%[e]
10a	Et	H	NO_2	38%	35%	N.T.[g]
10b	H	H	NO_2	10.7 ± 0.7[f]	79.4 ± 8.8[f]	16%
11a	Et	Cl	NH_2	20.4 ± 4.2	4.8 ± 0.5	84 ± 9
11b	H	Cl	NH_2	4.9 ± 0.44	3.6 ± 0.3	22.4 ± 2.8
12a	Et	H	NH_2	14%	16%	N.T.[g]
12b	H	H	NH_2	44%	35%	81 ± 5
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] Concentrations necessary for 50% inhibition (IC_{50}). The IC_{50} values were means ± SEM of three or four separate determinations in triplicate. [e] Reference 16. [f] Reference 15. [g] Not tested.

2-carboxylic acid **11b** is about only 4-fold more active than its corresponding ester **11a**, the 8-nitro-2-carboxylic acid **9b** shows about a 40-fold increased binding activity with respect to its corresponding ester **9a**.

Removal of the chlorine atom at position-7 of **1a,b** to give compounds **3a,b** causes a dramatic decrease in AMPA binding affinity, confirming the importance of the presence of electron-withdrawing groups at this position to obtain potent AMPA receptor antagonists. The same applies to compounds **10a,b** and **12a,b**[15] compared to **9a,b** and **11a,b**, respectively, although not to the same extent. In accordance with the reported

AMPA receptor pharmacophore model,[2,19] the crucial effect of the 7-chlorine atom is probably due to the increased acidity of the NH lactam proton.

It has to be noted that the presence of a proton-acceptor substituent at position-7 of the TQX framework, capable of engaging a hydrogen-bond receptor−ligand interaction, is well tolerated by the AMPA receptor.[2,18] In fact, the 7-(1,2,4-triazol-4-yl) derivatives **6a,b**, though lacking a substituent at position-8, show AMPA binding affinity comparable to that of some 7-chloro-8-heteroaryl derivatives. Moreover, the 7-(1,2,4-triazol-4-yl) derivatives **6a,b** show more than 4-fold increased binding affinity with respect to compounds **3a,b** bearing the heteroaromatic ring at the crucial position-8.

Affinities of the triazoloquinoxalines **1a,b**−**5a,b**, **7a,b**, and **8a,b** for the glycine/NMDA receptor are lower than those for the AMPA receptor. In fact, replacement of the 8-nitro group of the mixed Gly/NMDA and AMPA antagonists **9a,b** with a 8-heterocyclic substituent leads to a decrease in glycine/NMDA binding affinity, with the exceptions of the 8-(pyrrol-1-yl) **4b** and the 8-(pyrazol-1-yl) **7a,b** derivatives, which show affinities comparable to those of compounds **9a,b**. Thus, the presence of an 8-nitrogen-containing heterocycle, lacking the N^3-nitrogen atom, seems to be well-tolerated by the glycine/NMDA receptor. These data indicate that the pyrrole and pyrazole rings could be considered bioisosters of the nitro group for the anchoring of these derivatives at the glycine/NMDA receptor site. The same applies also to the amino group. In fact, the 8-amino substituted derivatives **11a,b** display comparable glycine/NMDA binding affinity with respect to the corresponding 8-nitro compounds **9a,b**.

Introduction of the 8-heterocyclic substituent leads, in general, to an increase in KA binding activity of the 2-carboxylic acid derivatives **1b**−**5b**,**7b**, and **8b** with respect to the inactive 8-nitro compound **9b**. However, all the reported 8-heteroaryl compounds show affinity in the micromolar range, **1b** and **5b** being the most active compounds toward the KA receptor. On the contrary, all the 8-heteroaryl-2-carboxyethyl derivatives **1a**−**5a,7a**, and **8a** are inactive at the KA receptor, with the only exception being **5a**. Replacement of the 8-nitro group of **9a,b** with an amino one gives compounds **11a,b**, which show an increased KA receptor affinity comparable to that of most of the 8-heteroaryl-2-carboxylic acid derivatives.

Removal of the chlorine atom at position-7 of the TQX framework causes a drastic reduction in glycine/NMDA binding affinity, thus confirming previously reported data.[15] In fact, compounds **10a,b** and **12a,b** are more than 10-fold less active than their corresponding 7-chloro derivatives **9a,b** and **11a,b**. To a minor extent, the same applies to KA receptor binding affinity (compare compounds **3b** and **12b** to **1b** and **11b**, respectively).

It has to be noted that the 2-carboxylic ester derivatives are in general less active than the corresponding acids toward the glycine/NMDA receptor, independent of the benzo-fused substitutions. These results confirm previously reported data on glycine/NMDA receptor antagonists.[15]

Some selected triazoloquinoxaline derivatives (**1b,4b**, **5b, 7b**, and **8b**) together with the well-known NBQX and DCKA were evaluated for functional antagonist activity by assessing their ability to inhibit depolarization induced by 5 μM AMPA or NMDA in mouse cortical wedge preparations. All the tested compounds inhibited AMPA and NMDA responses in a reversible manner (Table 2). The electrophysiological potencies of compounds **1b**, **5b**, and **8b** closely correlate with the results obtained in the AMPA and Gly/NMDA binding assays, thus confirming that these derivatives are selective AMPA antagonists. In fact, in agreement with [^{3}H]-AMPA and [^{3}H]glycine binding results, the inhibitory action of **1b**, **5b**, and **8b** on depolarization induced by 5 μM AMPA was higher than that on NMDA-evoked responses. On the contrary, the functional antagonist activity vs NMDA of the nonselective AMPA and Gly/NMDA antagonists **4b** and **7b** are not in agreement with the [^{3}H]glycine binding results. On the basis of [^{3}H]-AMPA and [^{3}H]glycine binding data, the respective values of the inhibitory action of **4b** on depolarization induced by 5 μM AMPA and NMDA were expected to be similar. Instead, the inhibitory action of this derivative on the depolarization induced by 5 μM AMPA was much higher than that on NMDA-evoked responses. Compound **7b** follows the same pattern.

To shed light on this contradictory data, the functional antagonism at the NMDA receptor-ion channel complex was demonstrated by the ability of compounds **4b** and **7b,** together with some other selected compounds (**1b**−**3b**, **5b**, **8b**, **9b**, and **11b**), to inhibit the binding of the channel blocking agent [^{3}H]-(+)-MK-801 ((+)-5-methyl-10,11-dihydro-5H-benzo[*a*,*d*]cyclohepten-5,10-imine maleate)[21−23] in rat cortical membranes incubated with 10μM glutamate and 0.1 μM glycine (Table 3). In general, the IC_{50} values of these compounds for glutamate-stimulated [^{3}H]-(+)-MK-801 binding closely correlated with their K_i values on [^{3}H]glycine binding. In

Table 2. Functional Antagonism at NMDA and AMPA Sites

	mouse cortical wedge preparation: IC_{50} (μM) vs agonist-induced depolarizations[a]	
compd	AMPA	NMDA
1b (TQX-173)[b]	2.3 ± 0.4	46 ± 4
4b	3.0 ± 0.3	52 ± 6.0
5b	1.2 ± 0.2	23 ± 3
7b	3.0 ± 0.4	150 ± 12
8b	12 ± 2	350 ± 30
NBQX	0.20 ± 0.02	c
DCKA	52 ± 11	4.7 ± 0.9

[a] Concentration that inhibits by 50% depolarizations (IC_{50}) induced by 5 μM AMPA or NMDA. The IC_{50} values were means ± SEM of four separate determinations. [b] Reference 16. [c] At 10 μM concentration the inhibition was not significant.

Table 3. Inhibition of Stimulated [^{3}H]-(+)-MK-801 Binding

compd	[^{3}H]-(+)-MK-801 IC_{50}[a] or *I*%[b]	compd	[^{3}H]-(+)-MK-801 IC_{50}[a] or *I*%[b]
1b (TQX-173)	68 ± 4 μM	**7b**	16.8 ± 1.4 μM
2b	45%	**8b**	30%
3b	20%	**9b**	17.8 ± 4.0 μM
4b	11.7 ± 0.7 μM	**11b**	5.6 ± 0.4 μM
5b	43 ± 2.0 μM		

[a] Concentration giving 50% inhibition of stimulated [^{3}H]-(+)-MK-801 binding: all assays were carried out in the presence of 10 μM glutamate and 0.1 μM glycine. The results were calculated from three or four separate determinations in triplicate. [b] Percentage of inhibition (*I*%) of specific binding at 100 μM concentration.

particular, the IC_{50} values of compounds **4b** and **7b** are in agreement with the results obtained in the [^{3}H]-glycine displacement assays.

In conclusion, this study has shown that the introduction of different heteroaromatic rings at position-8 of the TQX framework leads to other selective AMPA receptor antagonists. In fact, the herein reported data suggest that the presence of a N^3-nitrogen containing heterocycle at position-8 of the TQX framework is an essential feature for highly selective AMPA receptor antagonists.

We believe that these results could contribute to a greater understanding about the structural requirements of a ligand for the anchoring to the AMPA receptor in order to design novel potent and selective AMPA receptor antagonists.

On the basis of these new findings, further modifications of these TQX derivatives to improve biological activity and selectivity are in progress.

Experimental Section

Chemistry. Silica gel plates (Merck F_{254}) 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. 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. The following abbreviations are used: s = singlet, d = doublet, dd = double doublet, t = triplet, q = quartet, m = multiplet, br = broad, and ar = aromatic protons. Physical and analytical data of the synthesized compounds are listed in Table 4.

General Procedure To Prepare Ethyl 4,5-Dihydro-8-nitro-4-oxo-1,2,4-triazolo[1,5-*a*]quinoxaline-2-carboxylates 9a[16] **and 10a**. Ethyl 7-chloro-4,5-dihydro-4-oxo-1,2,4-triazolo[1,5-*a*]quinoxaline-2-carboxylate[15] or its 7-unsubstituted analogue[15] (0.65 mmol) was portionwise added to HNO_3 (90%, 2 mL). The solution was stirred at 0−5 °C until the disappearance of the starting material (TLC monitoring). The mixture was then poured onto ice (20 g) and the resulting solid was collected and washed with H_2O. Compound **9a** displayed the following spectral data: ^{1}H NMR (DMSO-d_6) 1.40 (t, 3H, CH_3), 4.47 (q, 2H, CH_2), 7.65 (s, 1H, ar), 8.81 (s, 1H, ar), 12.9 (br s, 1H, NH); IR 3170, 3060, 1715.

General Procedure To Prepare Ethyl 8-Amino-4,5-dihydro-4-oxo-1,2,4-triazolo[1,5-*a*]quinoxaline-2-carboxylates 11a[16] **and 12a**. Iron powder (5.2 g) was added to a solution of **9a** or **10a** (5.2 mmol) in glacial acetic acid (25 mL). The mixture was heated at 90 °C for 30 min. Evaporation at reduced pressure of the solvent yielded a residue which was dried and exhaustively Soxhlet extracted with acetone (500 mL). Most of the solvent was evaporated to yield a solid, which was collected. Compound **11a** displayed the following spectral data: ^{1}H NMR (DMSO-d_6) 1.38 (t, 3H, CH_3), 4.44 (q, 2H, CH_2), 5.8 (br s, 2H NH_2), 7.34 (s, 1H, ar), 7.60 (s, 1H, ar), 12.2 (br s, 1H, NH); IR 3400, 3260, 1700, 1640.

General Procedure To Prepare Ethyl 4,5-Dihydro-4-oxo-8-(1,2,4-triazol-4-yl)-1,2,4-triazolo[1,5-*a*]quinoxaline-2-carboxylates 1a[16] **and 3a**. Diformylhydrazine (4.0 mmol) and then, drop by drop, trimethylsilyl chloride (20 mmol) and triethylamine (9.3 mmol) were added to a suspension of **11a** or **12a** (1.33 mmol) in anhydrous pyridine (6 mL). The mixture was heated at 100 °C until the disappearance of the starting material (TLC monitoring). Evaporation at reduced pressure of the solvent yielded a solid that was treated with H_2O (6 mL), collected, and washed with H_2O. Compound **1a** was directly recrystallized, while compound **3a**, before recrystallization, was purified by column chromatography [eluting system $CHCl_3$/MeOH/AcOH (80:15:5)]. Compound **1a** displayed the following spectral data: ^{1}H NMR (DMSO-d_6) 1.37 (t, 3H, CH_3), 4.45 (q, 2H, CH_2), 7.70 (s, 1H, ar), 8.49 (s, 1H, ar), 8.95 (s, 2H, triazole H-3 and H-5), 12.7 (br s, 1H, NH); IR 3620, 3520, 3140, 3120, 1730, 1715.

Table 4. Physical and Analytical Data of the Synthesized Compounds

compd	mp, °C	cryst solv	% yield	C, H, N
1a[a]	>300	EtOH	73	$C_{14}H_{10}ClN_7O_3$
1b[a]	>300	EtOH	82	$C_{12}H_6ClN_7O_3$
2a[a]	275 dec	EtOH/AcOH	85	$C_{15}H_{11}ClN_6O_3$
2b[a]	>300	DMF	92	$C_{13}H_7ClN_6O_3$
3a	>300	EtOH/DMF	39	$C_{14}H_{11}N_7O_3$
3b	>300	DMF	97	$C_{12}H_7N_7O_3$
4a	>300	AcOH	80	$C_{16}H_{12}ClN_5O_3$
4b	250 dec	AcOH	88	$C_{14}H_8ClN_5O_3$
5a	230 dec	EtOH	80	$C_{16}H_{12}ClN_5O_4$
5b	228 dec	AcOH	68	$C_{14}H_8ClN_5O_4$
6a	>300	AcOH/H_2O	34	$C_{14}H_{11}N_7O_3$
6b	>300	AcOH/H_2O	80	$C_{12}H_7N_7O_3$
7a	>300	AcOH	96	$C_{15}H_{11}ClN_6O_3$
7b	263−265	EtOH/DMF	61	$C_{13}H_7ClN_6O_3$
8a	279 dec	EtOH	73	$C_{14}H_{10}ClN_7O_3$
8b	>300	EtOH/DMF	28	$C_{12}H_6ClN_7O_3$
9a[a]	283−285	EtOH	80	$C_{12}H_8ClN_5O_5$
9b[b]	>300	EtOH	68	$C_{10}H_4ClN_5O_5$
10a	288−300	EtOH	70	$C_{12}H_9N_5O_5$
10b[b]	237−240	EtOH	67	$C_{10}H_5N_5O_5$
11a[a]	>300	AcOH	44	$C_{12}H_{10}ClN_5O_3$
11b	>300	EtOH/H_2O	67	$C_{10}H_6ClN_5O_3$
12a	>300	EtOH/DMF	63	$C_{12}H_{11}N_5O_3$
12b	>300	EtOH/H_2O	41	$C_{10}H_7N_5O_3$
13[a]	208−210	EtOH	58	$C_9H_7ClN_4O_2$
14	170−172	EtOH/H_2O	86	$C_9H_7ClN_4O_2$
15	262−264	EtOH/DMF	89	$C_8H_6ClN_5O_2$
16[a]	172−174	EtOH	59	$C_{13}H_{11}Cl_2N_5O_4$
17	159−161	EtOH	63	$C_{13}H_{11}Cl_2N_5O_4$
18	182−184	EtOH/DMF	41	$C_{12}H_{10}Cl_2N_6O_4$
19[a]	235−237	EtOH	86	$C_{13}H_{13}ClN_6O_4$
20	180−182	EtOH	92	$C_{13}H_{13}ClN_6O_4$
21	206−208	dioxane/H_2O	77	$C_{12}H_{12}ClN_7O_4$
22	160−162	EtOH	91	$C_{17}H_{17}ClN_6O_7$
23	165−167	EtOH	82	$C_{16}H_{16}ClN_7O_7$
24[a]	177−179	EtOH	51	$C_{17}H_{15}ClN_6O_6$
25	136−138	EtOH	50	$C_{17}H_{15}ClN_6O_6$
26	175−177	EtOH	50	$C_{16}H_{14}ClN_7O_6$

[a] Reference 16. [b] Reference 15.

Ethyl 7-Chloro-4,5-dihydro-4-oxo-8-(pyrrol-1-yl)-1,2,4-triazolo[1,5-*a*]quinoxaline-2-carboxylate 4a. A solution of 2,5-diethoxytetrahydrofuran (2.43 mmol) in AcOH (5 mL) was dropwise added to a hot (90 °C) suspension of **11a** (0.81 mmol) in AcOH (10 mL). The mixture was kept at 90 °C for 5 min. Upon cooling a solid was obtained, which was collected and washed with EtOH: ^{1}H NMR (DMSO-d_6) 1.37 (t, 3H, CH_3), 4.44 (q, 2H, CH_2), 6.32 (t, 2H, pyrrole H-3 and H-4, J = 2.12 Hz), 7.12 (t, 2H, pyrrole H-2 and H-5, J = 2.12 Hz), 7.65 (s, 1H, ar), 8.00 (s, 1H, ar), 12.6 (br s, 1H, NH); IR 3300, 3115, 1750, 1710.

Ethyl 7-Chloro-4,5-dihydro-4-oxo-8-(3-formylpyrrol-1-yl)-1,2,4-triazolo[1,5-*a*]quinoxaline-2-carboxylate 5a. A solution of 2,5-dimethoxy-3-tetrahydrofurancarboxaldehyde (2.91 mmol) in AcOH (10 mL) was dropwise added to a suspension of **11a** (1.94 mmol) in AcOH (20 mL). The mixture was heated at 90 °C for 10 min. The mixture was cooled and diluted with H_2O (200 mL) to yield a solid, which was collected and washed with H_2O. A second crop of **5a** was obtained by extracting the mother liquor with ethyl acetate (3 × 50 mL). Evaporation of the dried (Na_2SO_4) organic layers afforded a solid, which was purified by column chromatography [eluting system $CHCl_3$/MeOH (90:10)]: ^{1}H NMR (DMSO-d_6) 1.35 (t, 3H, CH_3), 4.45 (q, 2H, CH_2), 6.71 (t, 1H, pyrrole H-4, J = 1.47 Hz), 7.27 (d, 1H, pyrrole H-5, J = 2.28 Hz), 7.69 (s, 1H, ar), 8.04 (s,

1H, pyrrole H-2), 8.24 (s, 1H, ar), 9.82 (s, 1H, CHO), 12.7 (br s, 1H, NH); IR 3300, 1750, 1670.

Ethyl 4,5-Dihydro-4-oxo-7-(1,2,4-triazol-4-yl)-1,2,4-triazolo[1,5-*a*]quinoxaline-2-carboxylates 6a. The title compound was prepared by reacting ethyl 7-amino-4,5-dihydro-4-oxo-1,2,4-triazolo[1,5-*a*]quinoxaline-2-carboxylate [15] (0.91 mmol) with diformylhydrazine (2.74 mmol), trimethylsilyl chloride (13.6 mmol), and triethylamine (6.2 mmol), as described above for the preparation of **1a** and **3a**:^{1}H NMR (DMSO-d_6) 1.37 (t, 3H, CH_3), 4.45 (q, 2H, CH_2), 7.60 (d, 1H, ar, J = 2.48 Hz), 7.71 (dd, 1H, ar, J = 8.8, 2.48 Hz), 8.30 (d, 1H, ar, J = 8.8 Hz), 9.18 (s, 2H, triazole protons), 12.7 (br s, 1H, NH); IR 3500, 3300, 1750, 1730.

4-Chloro-5-(imidazol-1-yl)-2-nitroaniline 13.[16,20] An excess of imidazole (38.4 mmol) and KOH (14.4 mmol) were added to a solution of 4,5-dichloro-2-nitroaniline (9.6 mmol) in anhydrous dimethylformamide (DMF) (20 mL). The mixture was heated at 80 °C for 1 h and then poured onto ice/water (50 mL). Extraction with ethyl acetate (200 mL × 2), anhydrification of the organic layers, and evaporation at reduced pressure of the solvent yielded a solid, which was treated with diethyl ether and collected: ^{1}H NMR (DMSO-d_6) 7.14 (d, 2H, ar + imidazole H-2), 7.49 (d, 1H, imidazole H-4, J = 1.22 Hz), 7.7 (br s, 2H, NH_2), 7.98 (d, 1H, imidazole H-5, J = 1.22 Hz), 8.22 (s, 1H, ar); IR 3400, 3300, 3150.

General Procedure To Prepare 4-Chloro-5-heteroaryl-2-nitroanilines 14 and 15. An excess of the suitable heterocycle (38.4 mmol) and KOH (14.4 mmol) were added to a solution of 4,5-dichloro-2-nitroaniline (9.6 mmol) in anhydrous DMF (20 mL). The mixture was heated at 100 °C for 6 h and then set aside at room temperature for 12 h. The resulting solid was collected and washed with a few drops of DMF. Compound **14** displayed the following spectral data: ^{1}H NMR (DMSO-d_6) 6.58 (dd, 1H, pyrazole H-4, J = 1.46, 2.56 Hz), 7.31 (s, 1H, ar), 7.7 (br s, 2H, NH_2), 7.82 (d, 1H, pyrazole H-3, J = 1.46 Hz), 8.19 (s, 1H, ar), 8.25 (d, 1H, pyrazole H-5, J = 2.56 Hz); IR 3450, 3350.

General Procedure To Prepare Ethyl N^1-[4-chloro-5-heteroaryl-2-nitrophenyl]idrazono-N^2-chloroacetates 16[16]**−18**. Concentrated H_2SO_4 (7 mL) was added dropwise to a suspension of **13**−**15** (4.19 mmol) in H_2O (10 mL). A solution of $NaNO_2$ (5%, 6 mL) was added to the cooled (0 °C) mixture, which was then stirred for 15 min to yield a solid that was quickly filtered off. A solution of $NaBF_4$ (17%, 4 mL) was added to the cold (0−5 °C) solution, which was allowed to stand in ice/water for 30 min. Methanol (25 mL) and ethyl 2-chloro-3-oxobutanoate (4.19 mmol) was added to the mixture, which was then stirred at room temperature for 3 h. Compounds **17** and **18** were obtained as solid and were collected and washed with H_2O. The reaction mixture containing compound **16** was instead diluted with H_2O (70 mL) and extracted with chloroform (60 mL × 3). Evaporation at reduced pressure of the dried (Na_2SO_4) organic solvent yielded a solid, which was treated with diethyl ether and collected. Compound **16** displayed the following spectral data: ^{1}H NMR (DMSO-d_6) 1.28 (t, 3H, CH_3), 4.33 (q, 2H, CH_2), 7.19 (d, 1H, imidazole H-4, J = 1.22 Hz), 7.62 (d, 1H, imidazole H-5, J = 1.22 Hz), 7.71 (s, 1H, ar), 8.10 (s, 1H, imidazole H-2), 8.53 (s, 1H, ar), 11.01 (s, 1H, NH); IR 3300, 1700.

General Procedure To Prepare Ethyl N^1-[4-chloro-5-heteroaryl-2-nitrophenyl]-N^2-oxamidrazonates 19[16]**−21**. Ammonia was bubbled until saturation into a stirred solution of **16**−**18** (0.81 mmol) in anhydrous dioxane (12 mL). The mixture was stirred at room temperature until the disappearance of the starting material (TLC monitoring). The mixture was then diluted with H_2O (50 mL) to yield a red solid, which was collected and washed with H_2O. Compound **19** displayed the following spectral data: ^{1}H NMR (DMSO-d_6) 1.24 (t, 3H, CH_3), 4.24 (q, 2H, CH_2), 6.9 (br s, 2H, NH_2), 7.15 (d, 1H, imidazole H-4, J = 1.22 Hz), 7.55 (d, 1H, imidazole H-5, J = 1.22 Hz), 7.69 (s, 1H, ar), 8.03 (s, 1H, imidazole H-2), 8.35 (s, 1H, ar), 10.04 (s, 1H, NH); IR 3480, 3360, 3300, 1760.

General Procedure To Prepare Ethyl N^1-(4-chloro-5-heteroaryl-2-nitrophenyl)-N^3-ethoxalyl-N^2-oxamidrazonates 22 and 23. Compound **20** or **21** (1.13 mmol) was dropwise added to a refluxing solution of ethyloxalyl chloride (2.26 mmol) in anhydrous toluene (70 mL). The resulting yellow solution was refluxed until the disappearance of the starting material (TLC monitoring). Upon cooling compound **23** precipitated as a yellow solid and was collected and washed with diethyl ether. Compound **22**, which does not precipitate, was isolated after evaporation of the solvent, treatment with diethyl ether, and filtration. Compound **22** displayed the following spectral data: ^{1}H NMR (DMSO-d_6) 1.23 (t, 3H, CH_3), 1.32 (t, 3H, CH_3), 4.25 (q, 2H, CH_2), 4.36 (q, 2H, CH_2), 6.64 (dd, 1H, pyrazole H-4, J = 1.47, 2.56 Hz), 7.90 (d, 1H, pyrazole H-3, J = 1.47 Hz), 7.98 (s, 1H, ar), 8.43 (d, 1H, pyrazole H-5, J = 2.56 Hz), 8.44 (s, 1H, ar), 10.9 (br s, 1H, NH), 13.0 (br s, 1H, NH); IR 3300, 1730, 1715.

Diethyl 1-[4-Chloro-5-(imidazol-1-yl)-2-nitrophenyl]-1,2,4-triazolo-3,5-dicarboxylate 24.[16] Compound **19** (1.13 mmol) was portionwise added to a boiling solution of ethyloxalyl chloride (2.26 mmol) in anhydrous toluene (50 mL). The resulting yellow solution was heated at reflux for 5 h. Elimination at reduced pressure of the solvent yielded a solid, which was dissolved in ethyl acetate (100 mL). The organic solution was washed with a solution of NaOH (0.5%, 40 mL × 2) and then with H_2O (40 mL). The dried (Na_2SO_4) organic layer was evaporated to afford a solid, which was treated with diethyl ether and collected: ^{1}H NMR (DMSO-d_6) 1.20 (t, 3H, CH_3), 1.33 (t, 3H, CH_3), 4.28 (q, 2H, CH_2), 4.40 (q, 2H, CH_2), 7.18 (d, 1H, imidazole H-4, J = 1.22 Hz), 7.64 (d, 1H, imidazole H-5, J = 1,22 Hz), 8.13 (s, 1H, imidazole H-2), 8.30 (s, 1H, ar), 8.80 (s, 1H, ar); IR 1750.

General Procedure To Prepare Diethyl 1-[4-Chloro-5-heteroaryl-2-nitrophenyl]-1,2,4-triazolo-3,5-dicarboxylates 25 and 26. Concentrated H_2SO_4 (4 mL) was dropwise added to finely powdered **22** and **23** (0.88 mmol) under stirring. The solution was stirred at room temperature for 12 h, poured onto ice (200 g), and finally extracted with ethyl acetate (2 × 100 mL). The organic layers were washed with a solution of $NaHCO_3$ (1%, 2 × 100 mL) and then with H_2O (100 mL). Evaporation of the dried (Na_2SO_4) solvent yielded a solid. Compound **25** displayed the following spectral data: ^{1}H NMR (DMSO-d_6) 1.19 (t, 3H, CH_3), 1.33 (t, 3H, CH_3), 4.28 (q, 2H, CH_2), 4.45 (q, 2H, CH_2), 6.67 (dd, 1H, pyrazole H-4, J = 1.47, 2.56 Hz), 7.92 (d, 1H, pyrazole H-3, J = 1.47 Hz), 8.33 (s, 1H, ar), 8.49 (d, 1H, pyrazole H-5, J = 2.56 Hz), 8.77 (s, 1H, ar); IR 1750, 1715.

General Procedure To Prepare Ethyl 7-Chloro-4,5-dihydro-8-heteroaryl-4-oxo-1,2,4-triazolo[1,5-*a*]quinoxaline-2-carboxylates 2a[16]**, 7a, and 8a**. The title compounds were obtained by reducing **24**−**26** (0.69 mmol) with iron powder (0.69 g) and AcOH (6 mL) following the procedure described above for the synthesis of **11a** and 1**2a**. Compound **2a** displayed the following spectral data: ^{1}H NMR (DMSO-d_6) 1.35 (t, 3H, CH_3), 4.44 (q, 2H, CH_2), 7.15 (d, 1H, imidazole H-4, J = 1.22 Hz), 7.54 (d, 1H, imidazole H-5, J = 1.22 Hz), 7.67 (s, 1H, ar), 8.0 (s, 1H, imidazole H-2), 8.20 (s, 1H, ar), 12.7 (br s, 1H, NH); IR 1750, 1715.

General Procedure To Prepare 4,5-Dihydro-4-oxo-1,2,4-triazolo[1,5-*a*]quinoxaline-2-carboxylic Acids 1b[16]**, 3b−8b, 9b, and 10b**[15]**.** A solution of NaOH (0.8 N, 25 mL) was added to a suspension of **1a, 3a**−**10a** (0.97 mmol) in EtOH (25 mL). The mixture was stirred at room temperature for 3 h. Acidification of the clear solution with HCl (6 N) yielded a solid, which was collected and washed with H_2O. Compound **1b** displayed the following spectral data: ^{1}H NMR (DMSO-d_6) 7.73 (s, 1H, ar), 8.44 (s, 1H, ar), 8.95 (s, 2H, triazole H-3 and H-5), 12.8 (br s, 1H, NH), 13.9 (br s, 1H, COOH); IR 3140, 3060, 1715.

7-Chloro-4,5-dihydro-8-(imidazol-1-yl)-4-oxo-1,2,4-triazolo[1,5-*a*]quinoxaline-2-carboxylic Acid 2b.[16] A solution of NaOH (0.8 N, 15 mL) was added to a suspension of **2a** (0.59 mmol) in EtOH (15 mL). The mixture was stirred at room temperature for 1 h. The resulting solid was collected and dissolved in a small volume of H_2O. The solution was acidified with AcOH to afford a suspension that was stirred for 30 min.

The solid was collected and washed with H_2O: ^1H NMR (DMSO-d_6) 7.14 (d, 1H, imidazole H-4, $J = 1.22$ Hz), 7.53 (d, 1H, imidazole H-5, $J = 1.22$ Hz), 7.65 (s, 1H, ar), 7.99 (s, 1H, imidazole H-2), 8.14 (s, 1H, ar), 12.6 (br s, 1H, NH); IR 3500, 3300, 1720, 1620.

General Procedure To Prepare 8-Amino-4,5-dihydro-4-oxo-1,2,4-triazolo[1,5-*a*]quinoxaline-2-carboxylic Acids 11b and 12b. A solution of NaOH (1N, 4 mL) was added to a suspension of **11a** and **12a** (0.65 mmol) in EtOH (3 mL). The mixture was heated at 90 °C for 2 h. Upon cooling the resulting jelly mixture was acidified with AcOH, stirred several hours, and then set aside overnight. The solid was collected and washed with H_2O. Compound **11b** displayed the following spectral data: ^1H NMR (DMSO-d_6) 5.65 (s, 2H, NH_2), 7.32 (s, 1H, ar), 7.53 (s, 1H, ar), 12.0 (s, 1H, NH); IR 3440, 3330, 3220, 3060, 1700, 1640.

Pharmacology. Binding Assay. Rat cortical synaptic membrane preparation and [^{3}H]glycine and [^{3}H]AMPA binding experiments were performed following described procedures.[12,24]

High-Affinity [^{3}H]Kainate Binding. Frozen membrane aliquots were resuspended (0.5 mg protein/mL) in 0.05 M Tris-citrate buffer, pH 7.4, and incubated at 37 °C for 30 min. The membranes were then washed with fresh ice-cold buffer by three centrifugation and resuspension cycles as described for [^{3}H]glycine and [^{3}H]AMPA binding assays. The final membrane pellets were then resuspended in buffer to give 0.2−0.3 mg of protein/400 μL. Binding assays were carried out in ice for 60 min in the presence of 5 nM [^{3}H]kainate (NEN Life Science Products, Boston, MA; specific activity 58 Ci/mmol) and tested compounds in a total volume of 0.5 mL. Nonspecific binding was assessed in the presence of 1 mM glutamate. Bound radioactivity was separated by rapid filtration through glass fiber paper (GF/C) in a Brandel harvester and washed with 3 × 5 mL ice-cold buffer.

Electrophysiological Assay. The cortical wedge preparation described by Mannaioni et al.[25] was used. Briefly, wedges obtained from white Swiss mice (male 15−25 g) were placed in a two-compartment bath so that most of the cortical tissue was contained in one chamber and the callosal tissue in the other. Silicone grease had been previously placed between the two portions of the incubation bath. The wedges were incubated at room temperature and perfused with Krebs solution (mM: NaCl, 135; $CaCl_2$, 2.4; KH_2PO_4, 1.3; $MgCl_2$, 1.2; $NaHCO_3$, 16.2; and glucose, 7.7), gassed with 95% O_2 and 5% CO_2 at a flow rate of 2 mL min^{-1}. After stabilization, the gray matter was perfused with a Mg^{2+}-free medium. AMPA (5 μM) and NMDA (5 μM) were repeatedly applied for 2 min, every 15 min, until response stabilization (control peaks). Starting from this moment, the wedges were continuously superfused with the antagonists, while AMPA and NMDA were applied every 15 min as described above. At the end of the assays, the wedges were washed out with antagonists-free Krebs solution and AMPA and NMDA applied again at 15-min intervals for 30−60 min to verify tissue response recovery. The variations of the dc potentials between the two compartments were monitored via Ag/AgCl electrodes and displayed on a chart recorder. The inhibitory potencies of the tested compounds were calculated by comparing the depolarization peaks obtained in the presence of both agonist and antagonist with control peaks.

[^{3}H]-(+)-MK-801 Binding. [^{3}H]-(+)-MK-801 binding assays were carried out according to the method of Yoneda and Ogita[26] with slight modification.

Rat cortical membranes were resuspended (0.5 mg protein/mL) in ice-cold 5 mM Tris-HCl buffer, pH 7.4, containing 0.08% v/v Triton X-100 and stirred for 10 min at 0−2 °C. They were then collected by centrifugation (48000*g* for 10 min) and submitted to four additional resuspension and centrifugation cycles before being finally resuspended in the appropriate volume of buffer (0.2−0.3 mg of protein/tube) for the binding assay. The assay incubations were carried out at room temperature for 120 min with 2.5 nM [^{3}H]-(+)-MK-801 (22.5 Ci/mmol), 10 μM glutamic acid, and 0.1 μM glycine in the presence and absence of the tested compound in a total volume of 0.5 mL. Bound radioactivity was separated by filtration through GF/C filters presoaked in 0.05% polyethylenimmine and washed with ice-cold buffer (3 × 5 mL). Nonspecific binding was determined in the presence of 100 μM phencyclidine hydrochloride.

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 on the basis of four to six scalar concentrations of the test compound in triplicate using the ALLFIT computer program[27] and, in the case of tritiated glycine and AMPA binding, converted to K_i values by application of the Cheng−Prusoff equation.[28] 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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