Synthesis and Biological Evaluation of a New Set of Pyrazolo[1,5-*c*]quinazoline-2-carboxylates as Novel Excitatory Amino Acid Antagonists

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In recent papers (Catarzi, D.; et al. *J. Med. Chem.* **1999**, *42*, 2478−2484; **2000**, *43*, 3824−3826; **2001**, *44*, 3157−3165) we reported the synthesis of a set of 4,5-dihydro-4-oxo-1,2,4-triazolo-[1,5-*a*]quinoxaline-2-carboxylates (TQXs) that were active at the Gly/NMDA and/or AMPA receptors. In the present work the synthesis and Gly/NMDA, AMPA, and KA receptor binding affinities of a set of 5,6-dihydro-5-oxo-pyrazolo[1,5-*c*]quinazoline-2-carboxylates **1a,b**−**4a,b**, **5a**, **6a**, and **7a,b**−**9a,b**, (±)-5,6-dihydro-pyrazolo[1,5-c]quinazoline-2,5-dicarboxylates **10a,b** and **11a,b**, and (±)-1,5,6,10b-tetrahydro-5-oxo-pyrazolo[1,5-*c*]quinazoline-2-carboxylates **12a,b**−**14a,b** are reported. The binding results indicate that compounds **1a,b**−**4a,b**, **5a**, **6a**, and **7a,b**−**9a,b** show good Gly/NMDA and/or AMPA receptor binding affinities, demonstrating that the pyrazoloquinazoline tricyclic system is an adequate alternative to the triazoloquinoxaline framework for anchoring at both receptor types. Moreover, the inactivity of the 2,5-dicarboxylate derivatives **10a,b** and **11a,b** at the Gly/NMDA and AMPA receptors indicates that the presence of a glycine moiety in the southern portion of the pyrazoloquinazoline framework is deleterious for receptor−ligand interaction. Finally, the binding data of compounds **12a,b**−**14a,b** indicate that lack of planarity in the northeastern region of the molecules shifts selectivity toward the Gly/NMDA receptor, depending on the benzofused substitutions. In general, the pyrazoloquinazoline derivatives herein reported were inactive at the KA receptor. A study of the functional antagonism at both the AMPA receptor and the NMDA receptor-ion channel complex was also performed on some selected compounds.

Introduction

Glutamate (Glu), the major excitatory neurotransmitter in the central nervous system, has the potential to influence the function of most neuronal circuits by interacting with disparate receptors that are classified as ionotropic (ion-channel forming) or metabotropic (second messenger coupled) receptors. The ionotropic glutamate receptors (iGluRs) are in their turn subdivided, according to their preferential synthetic agonists, into *N*-methyl-D-aspartate (NMDA), (*R,S*)-2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)propionic acid (AMPA), and kainate (KA) receptors.[1,2] The NMDA receptor possesses numerous binding sites, including the glutamate coagonist glycine binding site (Gly/NMDA).[3]

Overstimulation of iGluRs induces excitotoxic mechanisms and cell death, and it is involved in several neurodegenerative syndromes such as Parkinson's, Huntington's, and Alzheimer's disease, as well as in brain ischemia and epilepsy. Accordingly, competitive iGluR antagonists, as well as noncompetitive NMDA antagonists acting at the Gly/NMDA site, are of great interest for their therapeutic potential in the treatment of the above-mentioned neurological disorders.[1,4−9]

Chart 1. Structural Similarities and Differences for the Binding of TQX Derivatives to the Gly/NMDA and AMPA Receptors
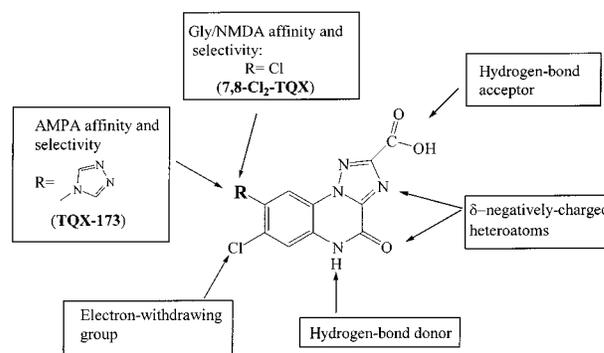
In the course of our efforts to find novel competitive and noncompetitive iGluR antagonists,[10−17] we have recently published works on a new set of 4,5-dihydro-4-oxo-1,2,4-triazolo[1,5-*a*]quinoxaline-2-carboxylates (TQXs) that are active at the Gly/NMDA and/or AMPA receptors.[14,15,17] Extensive structure−activity relationship (SAR) studies on the TQX series have provided further evidence of the structural similarities of each receptor recognition site.[4] As shown in Chart 1, some important common requirements for anchoring of these antagonists to the Gly/NMDA and AMPA receptor sites are (i) a NH proton donor that binds to a proton acceptor of

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Chart 2. Newly Synthesized Compounds

R = Et, Me, H
R_8 = H, Cl
R_9 = H, Cl, NO_2, NH_2, (1,2,4-triazol-4-yl)-, (3-formylpyrrol-1yl)
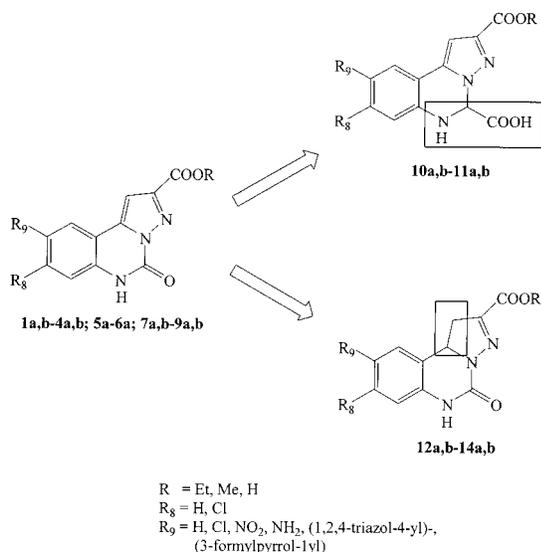
the receptors; (ii) the 3-nitrogen atom and the oxygen atom of the 4-carbonyl group that are δ-negatively charged heteroatoms able to form a Coulombic interaction with a positive site of the receptors; (iii) a carboxylate function at position-2 able to engage a strong hydrogen-bond interaction with a cationic proton donor site of the receptors; and (iv) an electron-withdrawing substituent, such as a chlorine atom, at position 7.[14] Furthermore, the SAR studies have also pointed out some different structural requirements for binding at the Gly/NMDA and AMPA receptors.[14,15,17] In particular, it has been clarified that diverse substituents at position-8 of the triazoloquinoxaline framework are able to distinguish the AMPA receptor from the Gly/NMDA site. In fact, the presence of a nitrogen-containing heterocycle at position-8, the best being the (1,2,4-triazol-4-yl) moiety (i.e., **TQX-173**, Chart 1), is required for potent and selective AMPA receptor antagonists.[15,17] On the other hand, the presence of a chlorine atom at the same position on the benzofused moiety is of paramount importance for Gly/NMDA receptor affinity and selectivity (i.e. **7,8-Cl₂TQX**, Chart 1).[14]

Encouraged by these results, we have now replaced the triazoloquinoxaline framework of the TQX compounds with a pyrazoloquinazoline ring system in order to investigate the influence of this structural modification on Gly/NMDA, AMPA, and KA receptor binding affinities. Thus, in this paper, we report the synthesis and binding affinities of some 5,6-dihydro-5-oxo-pyrazolo[1,5-*c*]quinazoline-2-carboxylates **1a,b−4a,b**, **5a**, **6a**, and **7a,b−9a,b** (Chart 2), which contain the important general requirements described above for the binding at each receptor type.

Moreover, with the aim of extending the knowledge on the structure−activity relationships of these tricyclic derivatives, further modifications on the pyrazoloquinazoline framework were performed. Thus, some (±)-5,6-dihydro-pyrazolo[1,5-*c*]quinazoline-2,5-dicarboxylates **10a,b**, **11a,b**, and some (±)-1,5,6,10b-tetrahydro-5-oxo-pyrazolo[1,5-*c*]quinazoline-2-carboxylates **12a,b−14a,b** were prepared and biologically evaluated (Chart 2). The effect of the introduction of a glycine moiety in the southern portion of the pyrazoloquinazoline framework was evaluated by synthesizing compounds **10a,b**

Scheme 1[a]

	R_4/R_8
15, 17, 19, 21, 1a, 10a	H
16, 18, 20, 22, 2a, 11a	Cl
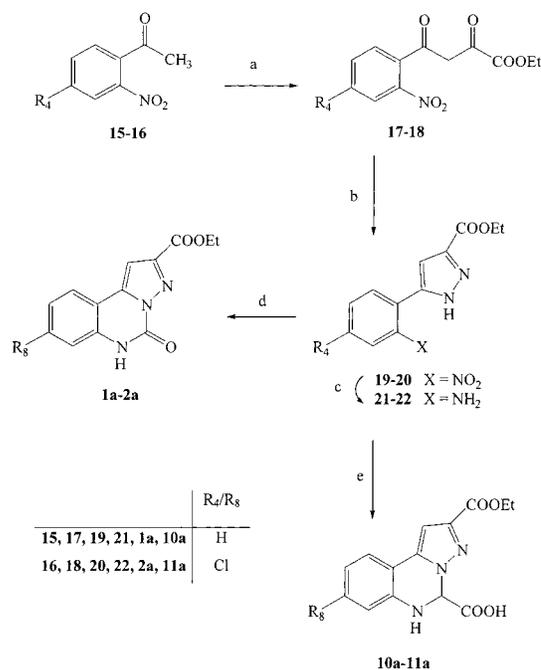
[a] Reagents: (a) diethyloxalate, EtONa; (b) 55% $N_2H_4 \cdot x H_2O$, 70% AcOH; (c) H_2/Pd/C; (d) $(CCl_3O)_2CO$, Et_3N; (e) CHOCOOH·H_2O.

and **11a,b**. In addition, compounds **12a,b−14a,b** were synthesized in order to investigate the effect of a lack of planarity in the northeastern region of the pyrazoloquinazoline moiety.

Chemistry

The synthesis of the pyrazolo[1,5-*c*]quinazoline-2-carboxylic esters **1a**[18,19]**−14a** and that of the 2-carboxylic acids **1b**[19,20]**−4b** and **7b−14b** are illustrated in Schemes 1−6.

Scheme 1 shows the synthetic pathway that yielded the 5,6-dihydro-5-oxo-pyrazolo[1,5-*c*]quinazoline-2-carboxylic esters **1a** and **2a**, and the (±)-5,6-dihydro-2-carboxyethyl-pyrazolo[1,5-*c*]quinazoline-5-carboxylic acids **10a** and **11a**. Briefly, by reacting the commercially available 2-nitroacetophenone **15** and its 4-chloro analogue **16**[21] with diethyloxalate, the ethyl 4-(2-nitroaryl)-2,4-dioxobutanoates **17**[22] and **18** were prepared. The latter were transformed into the ethyl 5(3)-(2-nitroaryl)-pyrazole-3(5)-carboxylates **19**[18] and **20** by reaction with hydrazine hydrate. Catalytic reduction (Pd/C) of the 2-nitroarylpyrazoles **19** and **20** afforded the corresponding amino derivatives **21**[18] and **22**, which were cyclized either with triphosgene or with glyoxylic acid to the final 5-oxo derivatives **1a** and **2a** and to the 5-carboxylic tricyclic analogues **10a** and **11a**, respectively.

Compounds **3a** and **4a** were prepared by reacting **1a** and **2a** with nitric acid (90%) (Scheme 2) and then transformed into the corresponding 9-amino derivatives **5a** and **6a** by reduction with iron in glacial acetic acid. Reaction of the 9-amino-8-chloro derivative **6a** with 1,2-diformylhydrazine yielded the 8-chloro-9-(1,2,4-triazol-4-yl) ester **8a**, while treatment of **6a** with 2,5-dimethoxy-3-tetrahydrofurancarboxyaldehyde gave the 8-chloro-9-(3-formylpyrrol-1-yl) ester **9a**.

The synthesis of the (±)-1,5,6,10b-tetrahydro-5-oxo-pyrazolo[1,5-*c*]quinazoline-2-carboxylic esters **12a−14a**

Scheme 2[a]

1a R$_8$ = H
2a R$_8$ = Cl

3a R$_8$ = H
4a R$_8$ = Cl

5a R$_8$ = H
6a R$_8$ = Cl

8a R$_9$ =

9a R$_9$ =
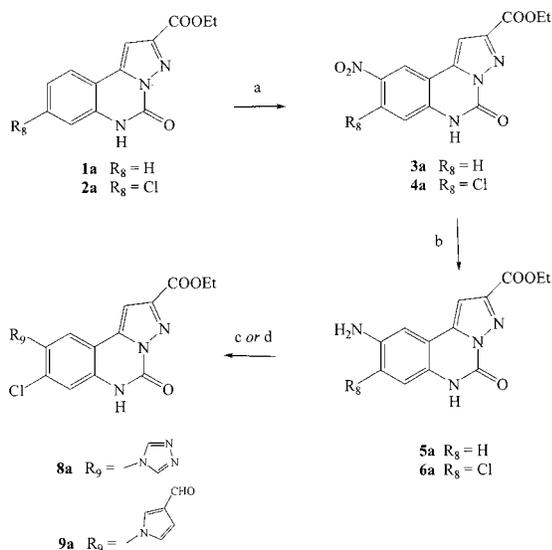
[a] Reagents: (a) 90% HNO$_3$; (b) iron, AcOH; (c) diformylhydrazine, Me$_3$SiCl, Et$_3$N, pyridine; (d) 2,5-dimethoxy-3-tetrahydrofurancarboxyaldehyde, AcOH.

Scheme 3[a]

23-25

26-28

29-31

32-34 X = NO$_2$
35-37 X = NH$_2$

12a-14a

	R$_4$/R$_8$	R$_5$/R$_9$
23, 26, 29, 32, 35, 12a	H	H
24, 27, 30, 33, 36, 13a	Cl	H
25, 28, 31, 34, 37, 14a	Cl	Cl
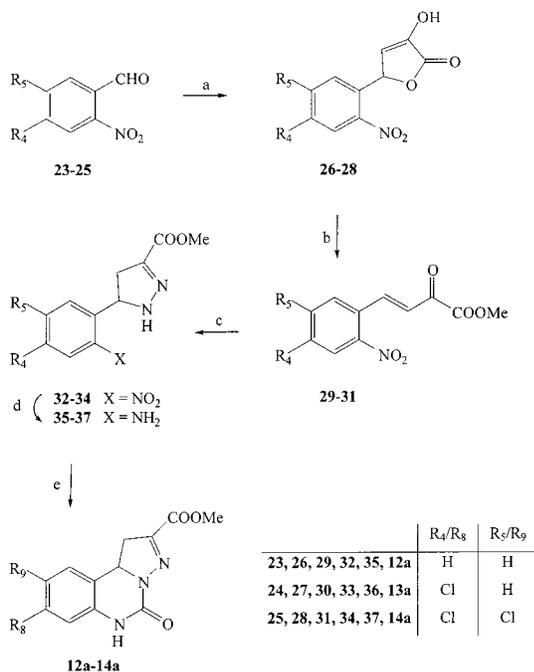
[a] Reagents: (a) CH$_3$COCOOH, HCl(g); (b) MeOH, HCl(g); (c) N$_2$H$_4$, EtOH; (d) H$_2$/PtO$_2$; (e) (CCl$_3$O)$_2$CO, Et$_3$N.

employed a totally different strategy, as shown in Scheme 3. Allowing the suitable *o*-nitrobenzaldehydes **23−25**[23,24] to react with pyruvic acid, using dry hydrogen chloride as catalyst, the corresponding 3-hydroxy-5-(2-nitroaryl)-5*H*-furan-2-ones **26**[25]**−28** were obtained. By refluxing compounds **26−28** in methanol, saturated with dry hydrogen chloride, the corresponding methyl 4-(2-nitroaryl)-2-oxo-3-butenoates **29**[25]**−31** were obtained, which with anhydrous hydrazine were converted into the methyl 4,5-dihydro-5-(2-nitroaryl)pyrazole-3-carboxylates **32−34**. Catalytic reduction (PtO$_2$) of **32−34** yielded the corresponding amino derivatives **35−37**, which were transformed into the final tricyclic derivatives **12a-14a** with triphosgene. Treatment of **12a** and

Scheme 4[a]

14a

7a
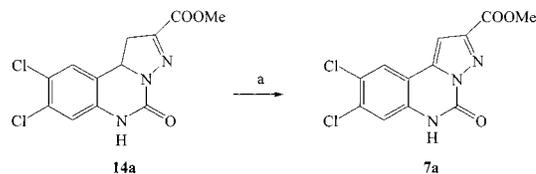
[a] Reagents: (a) tetrachloro-1,2-benzoquinone.

Scheme 5[a]

1a-4a, 7a-9a

1b-4b, 7b-9b

12a-14a

12b-14b
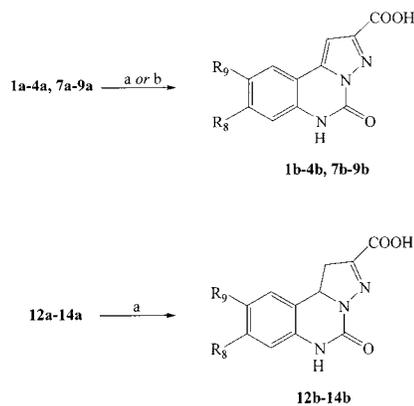
	R$_8$	R$_9$
1b, 12b	H	H
2b, 13b	Cl	H
3b	H	NO$_2$
4b	Cl	NO$_2$
7b, 14b	Cl	Cl
8b	Cl	
9b	Cl	

[a] Reagents: (a) AcOH, 6 N HCl; (b) 1.5 N KOH/AcOH or 6 N HCl.

Scheme 6[a]

21-22

38-39

10b-11b

	R$_4$/R$_8$
21, 38, 10b	H
22, 39, 11b	Cl
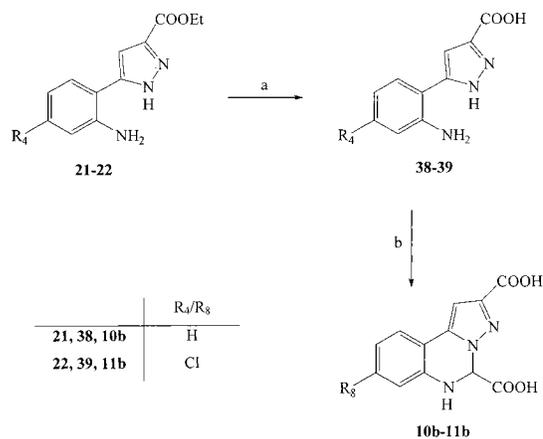
[a] Reagents: (a) 3 N NaOH/AcOH; (b) CHOCOOH·H$_2$O.

13a with nitric acid (90%) to obtain the corresponding 9-nitro derivatives afforded, in both cases, a mixture containing prevalently the corresponding 9-nitro dehydro derivatives **3a** and **4a**, respectively (by ^{1}H NMR analysis).

Dehydrogenation of compound **14a** with tetrachloro-1,2-benzoquinone gave the methyl 8,9-dichloro-5,6-dihydro-5-oxo-pyrazolo[1,5-*c*]quinazoline-2-carboxylate **7a** (Scheme 4).

Table 1. Displacement of [³H]Glycine, [³H]AMPA, and [³H]KA Binding[a]

1a,b–4a,b; 5a–6a; 7a,b–9a,b   **10a,b–11a,b**   **12a,b–14a,b**

compd	R	R_8	R_9	K_i (µM)[b] or I%[c] [³H]glycine	[³H]AMPA	IC$_{50}$ (µM)[d] or I%[c] [³H]KA
1a	Et	H	H	33.3 ± 7	42 ± 6.8	12%
1b	H	H	H	1.41 ± 0.3	12.4 ± 2.5	26%
2a	Et	Cl	H	26.5 ± 4.4	72 ± 2.3	15%
2b	H	Cl	H	0.48 ± 0.04	2.3 ± 0.4	60 ± 3
3a	Et	H	NO₂	15%	27%	4%
3b	H	H	NO₂	35%	26 ± 9	24%
4a	Et	Cl	NO₂	10.6 ± 1.8	8.2 ± 2	23%
4b	H	Cl	NO₂	1.1 ± 0.1	0.74 ± 0.04	23 ± 2
5a	Et	H	NH₂	30%	27%	30%
6a	Et	Cl	NH₂	40%	33%	11%
7a	Me	Cl	Cl	1.3 ± 0.2	42%	10%
7b	H	Cl	Cl	0.16 ± 0.04	2.4 ± 0.8	41 ± 3
8a	Et	Cl	(N-methylimidazolyl)	18%	0.87 ± 0.18	38%
8b	H	Cl	(N-methylimidazolyl)	8.3 ± 2.0	0.14 ± 0.02	5.1 ± 1.4
9a	Et	Cl	(HOOC-N-methylpyrrolyl)	57 ± 9	1.4 ± 0.2	29%
9b	H	Cl	(HOOC-N-methylpyrrolyl)	8.2 ± 3	0.27 ± 0.02	5.5 ± 0.4
10a	Et	H	H	15%	12%	0%
10b	H	H	H	35%	0%	11%
11a	Et	Cl	H	5%	6%	1%
11b	H	Cl	H	25%	7%	13%
12a	Me	H	H	40%	29%	0%
12b	H	H	H	2.0 ± 0.4	96 ± 8	19.5 ± 0.5
13a	Me	Cl	H	10.6 ± 3.9	17.3 ± 4.1	3%
13b	H	Cl	H	0.24 ± 0.03	7.2 ± 0.6	20%
14a	Me	Cl	Cl	4.0 ± 1.0	8.5 ± 1.3	9%
14b	H	Cl	Cl	0.19 ± 0.02	2.65 ± 0.48	17%
7,8-Cl₂-TQX[e]				0.074 ± 0.003	1.3 ± 0.2	NT[f]
TQX-173[g]				33.5 ± 5.3	0.14 ± 0.02	11.6 ± 1.3

[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 14. [f] Not tested. [g] Reference 15.

Finally, the 5,6-dihydro-pyrazoloquinazoline esters **1a–4a**, **7a–9a**, and the 1,5,6,10b-tetrahydro-pyrazolo-quinazoline esters **12a–14a** were hydrolyzed to their corresponding acids **1b–4b**, **7b–9b**, and **12b–14b**, respectively (Scheme 5).

Hydrolysis of the 2-ethyl ester of (±)-5,6-dihydro-pyrazolo[1,5-*c*]quinazoline-2,5-dicarboxylic acids **10a** and **11a**, either in acidic or alkaline media, afforded the corresponding acids **10b** and **11b** with very low yield. Thus, compounds **10b** and **11b** were obtained by following a different procedure (Scheme 6). Alkaline hydrolysis of the 5-(2-aminoaryl)pyrazole esters **21** and **22** gave the corresponding acids **38** and **39**, which were cyclized to the final derivatives **10b** and **11b** with glyoxylic acid.

Results and Discussion

The pyrazoloquinazolines **1a,b–4a,b**, **5a**, **6a**, and **7a,b–14a,b** were tested for their ability to displace tritiated glycine, AMPA, and KA from their specific binding sites in rat cortical membranes. The binding data are shown in Table 1 together with those of

previously reported **7,8-Cl₂-TQX**[14] and **TQX-173**[15] included as reference compounds.

In general, the results listed in Table 1 indicate that the 5,6-dihydro-5-oxo-pyrazolo[1,5-*c*]quinazoline-2-carboxylates **1a,b–4a,b**, **5a**, **6a**, and **7a,b–9a,b** show good Gly/NMDA and/or AMPA receptor binding affinities.

In particular, the binding data indicate that all the 2-carboxylic acids **1b–4b** and **7b–9b** are more active than their corresponding esters **1a–4a** and **7a–9a** at the Gly/NMDA and AMPA receptors. These data confirm that the presence in tricyclic derivatives of a 2-anionic carboxylate residue, able to engage a strong interaction with a cationic proton donor site of the receptors, is important for anchoring at both receptor sites.[14,15,17,26]

The unsubstituted ethyl ester **1a** displays comparable Gly/NMDA (K_i = 33.3 µM) and AMPA receptor affinity (K_i = 42 µM), while its corresponding 2-carboxylic acid **1b** is about 9-fold more active at the Gly/NMDA receptor (K_i= 1.41 µM) than at the AMPA one (K_i = 12.4 µM).

Introduction of different substituents on the benzo-fused moiety of the parents **1a,b** variously affects the

receptor−ligand interaction. In fact, the presence of a chlorine atom at position-8 (compounds **2a,b**) has diverse effects on binding activities. While the 2-carboxylic ester **2a** shows a comparable binding affinity to that of **1a** at both receptors, the corresponding acid **2b** is 3- and 6-fold more active than **1b** at the Gly/NMDA and AMPA receptors, respectively. Instead, the presence of a 9-nitro (**3a,b**) and a 9-amino group (**6a**) dramatically decreases Gly/NMDA and AMPA receptor affinities with respect to those of **1a,b**, with the only exception being compound **3b**, which is only 2-fold less active than compound **1b** at the AMPA receptor.

In addition, the presence of different substituents at position-9, holding constant the 8-chloro-substitution pattern, produces different effects on Gly/NMDA or AMPA binding activities, depending on the nature of the 9-substituent. In fact, while introduction of a 9-amino group is deleterious for the anchoring at both receptors (see **6a** vs **2a**), the presence of a 9-nitro substituent is well-tolerated, especially for the binding to the AMPA receptor (see **4a,b** vs **2a,b**, respectively). Introduction of a chlorine atom at position-9 of compound **2b** affords **7b**, which is about 3-fold more selective than **2b** for the Gly/NMDA receptor. In fact, **7b** is 15-fold more active at the Gly/NMDA receptor than at the AMPA one and is the most active Gly/NMDA antagonist herein reported. Furthermore, the 8,9-dichloro substituted methyl ester **7a** is one of the most selective Gly/NMDA receptor ligands herein reported. Introduction of a heteroaryl substituent at position-9 of compounds **2a,b** yields compounds **8a,b** and **9a,b**, which, as expected on the basis of previous findings on the TQX series,[15,17] are selective AMPA receptor ligands, being 30−60-fold more active at the AMPA receptor than at the Gly/NMDA one.

Finally, the pyrazoloquinazolines **1a,b-4a,b**, **5a**, **6a**, and **7a,b−9a,b** are in general inactive or active in the high micromolar range at the KA receptor with the only exceptions being the 2-carboxylic acids **8b** and **9b**, which display IC_{50} values of about 5 μM. These data confirm that the presence of a free carboxylic group at position-2 together with an 8-chloro substituent and a 9-heteroaryl group seems to be of paramount importance for KA receptor−ligand interaction.[15]

To summarize, these results are in accordance with previous findings on the TQX series and indicate that the pyrazoloquinazoline framework could replace the triazoloquinoxaline one for anchoring to the Gly/NMDA and AMPA receptors. In fact, the 8,9-dichloro acid **7b** shows a comparable affinity and the same degree of selectivity for the Gly/NMDA receptor with respect to the reference **7,8-Cl₂TQX** (Table 1). Moreover, compound **8b**, bearing the claimed (1,2,4-triazol-4-yl) group on the benzofused moiety, is equiactive to the reference **TQX-173** at the AMPA receptor, although about 4-fold less selective.

On this basis we performed some structural modifications on the pyrazoloquinazoline framework in order to further investigate the SAR of these tricyclic derivatives. Thus we synthesized the (±)-5,6-dihydro-pyrazolo[1,5-*c*]quinazoline-2,5-dicarboxylates **10a,b**, **11a,b**, and the (±)-1,5,6,10b-tetrahydro-5-oxo-pyrazolo[1,5-*c*]quinazoline-2-carboxylates **12a,b−14a,b** (Table 1).

Compounds **10a,b** and **11a,b** were formally obtained by substitution with a glycine moiety of the lactam group at position 5, 6 of **1a,b** and **2a,b**, respectively. In fact, a glycine moiety in the southern portion of the molecules is considered an important requirement for highly potent and selective bicyclic Gly/NMDA receptor antagonists,[27,28] and to our knowledge, it has never been introduced into tricyclic heteroaromatic derivatives in order to obtain Gly/NMDA, AMPA, or KA receptor antagonists. The inactivity of compounds **10a,b** and **11a,b** at all the three receptors indicates that a glycine moiety is deleterious for the anchoring of pyrazoloquinazolines to the receptor sites. In fact, the replacement of the 5,6-lactam group with a glycine moiety could change the binding mode of the molecules, making other pharmacophore portions, such as the important 2-carboxylate function, not optimized.

Compounds **12a,b−14a,b** were synthesized in order to evaluate the importance of the lack of planarity in the northeastern region of the pyrazoloquinazoline derivatives in receptor ligand interaction. In fact, the northern region of tricyclic and bicyclic iGluR antagonists was targeted for increasing the selectivity for either AMPA or Gly/NMDA receptors.[4]

The binding results indicate that compounds **12a,b−14a,b**, like the planar analogues (**1a,b−4a,b**, **5a**, **6a**, and **7a,b−9a,b**), are inactive at the KA receptor, while they show good Gly/NMDA and/or AMPA binding affinities. In particular, the 2-carboxylic acid derivatives **12b−14b** show higher affinities at both receptors than their corresponding esters **12a−14a** and are about 15−50-fold more active at the Gly/NMDA receptor than at the AMPA one. Moreover, the presence of a chlorine atom at position-8 of the benzofused moiety significantly increases the binding affinity at both receptors (see **13a,b** vs **12a,b**, respectively). Introduction of a chlorine atom at position-9 of the 8-chloro derivatives **13a,b** exerts different effects on the binding activities. In fact, the 8,9-dichloro-substituted methyl ester **14a** is about 2-fold more active than its 8-chloro analogue **13a** at both receptors. On the contrary, the 8,9-dichloro-substituted 2-carboxylic acid **14b** is about 3-fold more active than **13b** at the AMPA receptor, while it shows comparable Gly/NMDA receptor affinity.

Comparison of the binding affinities of the (±)-1,5,6,-10b-tetrahydro derivatives **12b**, **13b**, and **14a,b** with those of their planar analogues **1b**, **2b**, and **7a,b**, respectively, indicates that the lack of planarity in the northeastern region of the pyrazoloquinazoline tricyclic system does not significantly influence Gly/NMDA binding affinity, while it affects the anchoring to the AMPA receptor depending on the benzofused substitutions. In fact, the unsubstituted **12b** and its 8-chloro analogue **13b** are less active at the AMPA receptor than **1b** and **2b**, respectively, while they show comparable Gly/NMDA binding affinities. Thus **12b** and **13b** are about 6-fold more selective for the Gly/NMDA receptor than **1b** and **2b**, respectively. On the contrary, the 8,9-dichloro substituted methyl ester **14a** is significantly more active than its planar analogue **7a** at the AMPA receptor, while it is only 3-fold more active at the Gly/NMDA receptor. Thus, **14a** is a nonselective Gly/NMDA receptor antagonist. Finally, the 8,9-dichloro-substituted acid **14b** is as selective as **7b** toward the Gly/NMDA

Table 2. Inhibition of Stimulated [³H]-(+)-MK-801 Binding

compd	[³H]-(+)-MK-801 IC$_{50}$ (μM)[a] or I%[b]	compd	[³H]-(+)-MK-801 IC$_{50}$ (μM)[a] or I%[b]
1a	46 ± 4	**8b**	13.4 ± 1.1
1b	10.4 ± 1.4	**9a**	34%
2a	92 ± 8	**9b**	17 ± 2.0
2b	1.8 ± 0.2	**12b**	17.5 ± 1.4
4a	16.2 ± 2.1	**13a**	16.1 ± 1.1
4b	4.33 ± 0.4	**13b**	2.1 ± 0.09
7a	2.4 ± 0.3	**14a**	6.0 ± 0.46
7b	0.89 ± 0.04	**14b**	0.79 ± 0.05
8a	91 ± 8	**7,8-Cl$_2$-TQX**[c]	0.30 ± 0.05

[a] Concentration giving 50% inhibition of stimulated [³H]-(+)-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. [c] Reference 14.

Table 3. Functional Antagonism at AMPA and NMDA Sites

	mouse cortical wedge preparation: IC$_{50}$ (μM) vs agonist-induced depolarizations[a]	
compd	AMPA	NMDA
8a	1.75 ± 0.25	>50
8b	0.5 ± 0.07	20 ± 2.0
9a	1.0 ± 0.2	>50
9b	0.5 ± 0.1	25 ± 4
TQX-173[b]	2.3 ± 0.4	46 ± 4

[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 15.

receptor, because of their similar binding affinities not only at the Gly/NMDA but also at the AMPA receptor.

Functional antagonism at the NMDA receptor-ion channel complex was demonstrated by the ability of some pyrazoloquinazolines (**1a,b**, **2a,b**, **4a,b**, **7a,b**–**9a,b**, **12b**, **13a,b**, and **14a,b**) to inhibit binding of the channel blocking agent [³H]-(+)-MK-801 [(+)-5-methyl-10,11-dihydro-5*H*-benzo[*a,d*]cyclohepten-5,10-imine maleate][28−30] in rat cortical membranes incubated with 10 μM glutamate and 0.1 μM glycine. The results are listed in Table 2. In general, the IC$_{50}$ values of these compounds for glutamate stimulated [³H]-(+)-MK-801 binding are closely correlated with their K_i values on [³H]glycine binding. In particular, as in the case of [³H]-glycine displacement assays, compounds **7b** and **14b**, bearing chlorine atoms at positions 8 and 9, are the most potent compounds tested, with an IC$_{50}$ of 0.89 ± 0.04 and 0.79 ± 0.05 μM, respectively. These values are very close to that obtained with the reference **7,8-Cl$_2$-TQX**.[14]

Furthermore, compounds **8a,b** and **9a,b** were evaluated for functional antagonist activity at both the AMPA receptor and NMDA receptor-ion channel complex by assessing their ability to inhibit depolarization induced by 5 μM AMPA or 5 μM NMDA in mouse cortical wedge preparations (Table 3). All the tested compounds inhibited AMPA and NMDA responses in a reversible manner. The results obtained in the electrophysiological assay confirm that compounds **8a,b** and **9a,b**, bearing a heteroaryl substituent at position-9 of the benzofused moiety, are potent and selective AMPA receptor antagonists. In fact, in agreement with [³H]AMPA and [³H]-glycine binding data, the inhibitory actions of **8a,b** and **9a,b** on depolarization induced by 5 μM AMPA are much higher than those on NMDA-evoked response.

Morever, compounds **8a,b** and **9a,b** are more potent AMPA receptor antagonists than the reference **TQX-173**.[15]

In conclusion, this study has shown that the pyrazoloquinazoline tricyclic system is an adequate alternative to the TQX framework for anchoring to the Gly/NMDA and AMPA receptors. Moreover, the present work has evidenced that introduction of a glycine moiety in the southern portion of the pyrazoloquinazoline tricyclic system is deleterious for receptor−ligand interaction and that the lack of planarity in the northeastern region of the molecules could shift selectivity toward the Gly/NMDA receptor, depending on the benzofused substitutions.

On the basis of these preliminary findings, further modifications on the pyrazoloquinazoline tricyclic system are in progress to improve biological activity and selectivity.

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, 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 ¹H 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. The physical and analytical data of the newly synthesized compounds are shown in Table 4.

Materials. Commercially available starting materials were utilized as such, while the following products were prepared according to reported methods: **1a**,[18] **1b**,[20] **16**,[21] **17**,[22] **19**,[18] **21**,[18] **24**,[23] and **25**.[24]

Ethyl 4-(4-Chloro-2-nitrophenyl)-2,4-dioxobutanoate (18). Diethyl oxalate (20 mmol) and then 4-chloro-2-nitroacetophenone (**16**)[21] (20 mmol) were added portionwise into a cold (−5 °C) solution of sodium ethoxide (0.46 g of Na in 7 mL of absolute ethanol). The reaction mixture was allowed to stand at room temperature for 12 h and was then diluted with cold water (200 mL) and filtered. The clear mother liquor was acidified with glacial acetic acid to afford a suspension that was set aside at 4 °C for 6 h. The solid obtained was collected and washed with water: ¹H NMR (DMSO-d_6) 1.26 (t, 3H, CH$_3$, J = 7.0 Hz), 4.25 (q, 2H, CH$_2$, J = 7.0 Hz), 6.38 (s,1H, exchangeble with D$_2$O), 7.74 (d, 1H, ar, J = 8.2 Hz), 7.94 (d, 1H, ar, J = 8.2 Hz), 8.26 (s, 1H, ar); IR 1650, 1750.

Ethyl 5(3)-(4-Chloro-2-nitrophenyl)pyrazole-3(5)-carboxylate (20). Hydrazine hydrate (55%, 2.3 mmol) was added to a suspension of **18** (2.3 mmol) in glacial acetic acid (70%, 10 mL). The mixture was heated at 85 °C for 1 h. Evaporation at reduced pressure of the solvent afforded an oily residue that became solid upon treatment with cyclohexane: ¹H NMR (DMSO-d_6) 1.31 (t, 3H, CH$_3$, J = 7.0 Hz), 4.33 (q, 2H, CH$_2$, J = 7.0 Hz), 7.21 (s, 1H, pyrazole H-4), 7.80 (d, 1H, ar, J = 8.6 Hz), 7.86 (d, 1H, ar, J = 8.6 Hz), 8.09 (s, 1H, ar); IR 1725, 3240.

Ethyl 5(3)-(2-Amino-4-chlorophenyl)pyrazole-3(5)-carboxylate (22). A mixture of **20** (1.3 mmol) and 30% (w/w) Pd/C (10%) in ethyl acetate (50 mL) was hydrogenated in a Parr apparatus at 30 psi for 12 h. Elimination of the catalyst and evaporation at reduced pressure of the solvent yielded a solid that was treated with diethyl ether and collected: ¹H NMR (DMSO-d_6) 1.33 (t, 3H, CH$_3$, J = 7.0 Hz), 4.34 (q, 2H, CH$_2$, J

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

compd	mp (°C)	solvent[a]	yield (%)	C, H, N
2a	306−309	A	90	$C_{13}H_{10}ClN_3O_3$
2b	>300	B	98	$C_{11}H_6ClN_3O_3$
3a	>300	C	85	$C_{13}H_{10}N_4O_5$
3b	>300	B	90	$C_{11}H_6N_4O_5$
4a	>300	A	85	$C_{13}H_9ClN_4O_5$
4b	>300	D	90	$C_{11}H_5ClN_4O_5$
5a	>300	E	30	$C_{13}H_{12}N_4O_3$
6a	>300	A	65	$C_{13}H_{11}ClN_4O_3$
7a	>300	C	85	$C_{12}H_7Cl_2N_3O_3$
7b	>300	D	80	$C_{11}H_5Cl_2N_3O_3$
8a	>300	B	60	$C_{15}H_{11}ClN_6O_3$
8b	>300	B	90	$C_{13}H_7ClN_6O_3$
9a	295−296	A	70	$C_{18}H_{13}ClN_4O_4$
9b	>300	A	65	$C_{16}H_9ClN_4O_4$
10a	206−208	A	80	$C_{14}H_{13}N_3O_4$
10b	240−242	A	40	$C_{12}H_9N_3O_4$
11a	242−244	A	95	$C_{14}H_{12}ClN_3O_4$
11b	>300 dec	A	65	$C_{12}H_8ClN_3O_4$
12a	281−284	F	75	$C_{12}H_{11}N_3O_3$
12b	252−255	D	55	$C_{11}H_9N_3O_3$
13a	276−278	A	90	$C_{12}H_{10}ClN_3O_3$
13b	262−264	D	60	$C_{11}H_8ClN_3O_3$
14a	273−275	A	75	$C_{12}H_9Cl_2N_3O_3$
14b	269−271	D	95	$C_{11}H_7Cl_2N_3O_3$
18[b]	91−95	G	45	$C_{12}H_{10}ClNO_6$
20	137−139	H	95	$C_{12}H_{10}ClN_3O_4$
22	184−186	H	95	$C_{12}H_{12}ClN_3O_2$
26[c]	140−142[d]	I	70	
27	158−160[d]	I	90	
28	169−171[d]	I	70	
29[e]	90−92	J	65	$C_{11}H_9NO_5$
30[f]	98−99	J	40	$C_{11}H_8ClNO_5$
31[g]	116−118	J	30	$C_{11}H_7Cl_2NO_5$
32	121−122	A	90	$C_{11}H_{11}N_3O_4$
33	138−140	A	40	$C_{11}H_{10}ClN_3O_4$
34	151−153	A	70	$C_{11}H_9Cl_2N_3O_4$
35	111−115[d]	I	80	
36	119−123[d]	I	75	
37	139−143 dec[d]	I	77	
38	265−267	D	90	$C_{10}H_9N_3O_2$
39	278−280	D	80	$C_{10}H_8ClN_3O_2$

[a] Recrystallization solvents: A = ethanol, B = dimethylformamide, C = glacial acetic acid, D = dimethylformamide/water, E = nitromethane, F = acetonitrile, G = cyclohexane, H = cyclohexane/ethyl acetate, I = instable upon recrystallization, J = methanol. [b] Calcd (found) C, 48.09 (47.75); H, 3.37 (3.38); N, 4.67 (4.72). [c] Lit. mp 138−139 °C. [d] Melting point of the crude product. [e] Calcd (found) C, 56.17 (56.49); H, 3.86 (3.82); N, 5.96 (6.01). [f] Calcd (found) C, 48.99 (48.71); H, 3.00 (3.09); N, 5.20 (5.16). [g] Calcd (found) C, 43.44 (43.21); H, 2.32 (2.35); N, 4.61 (4.56).

= 7.0 Hz), 6.55 (br s, 3H, 1 ar + NH$_2$), 6.80 (s, 1H, ar), 7.30 (s, 1H, pyrazole H-4), 7.60 (d, 1H, ar, J = 8.0 Hz); IR 1740, 3320.

Ethyl 8-Chloro-5,6-dihydro-5-oxo-pyrazolo[1,5-*c*]quinazoline-2-carboxylate (2a). Triphosgene (0.37 mmol) and triethylamine (2.3 mmol) were successively added to a solution of **22** (0.94 mmol) in anhydrous tetrahydrofuran (10 mL). The mixture was stirred at room temperature for 30 min and then diluted with water (30 mL) to yield a solid, which was collected: ^{1}H NMR (DMSO-d_6) 1.33 (t, 3H, CH$_3$, J = 7.0 Hz), 4.36 (q, 2H, CH$_2$, J = 7.0 Hz), 7.33−7.38 (m, 2H, ar), 7.70 (s, 1H, H-1), 8.17 (d, 1H, ar, J = 8.9 Hz); IR 1710, 1740, 3240.

General Procedure To Prepare the 2-Ethyl Ester of (±)-5,6-Dihydro-pyrazolo[1,5-*c*]quinazoline-2,5-dicarboxylic Acids (10a and 11a). An excess of glyoxylic acid monohydrate (1.3 mmol) was added to a solution of **21**[18]−**22** (0.86 mmol) in anhydrous tetrahydrofuran (20 mL). The mixture was refluxed under nitrogen atmosphere. The reaction was monitored by TLC (eluting system CHCl$_3$/MeOH/AcOH 8:0.5:0.5) and the heating was continued until the starting material disappeared. Evaporation at reduced pressure of the solvent afforded an oily residue that became solid upon treatment with cyclohexane. Compound **11a** displayed the

following spectral data: ^{1}H NMR (DMSO-d_6) 1.30 (t, 3H, CH$_3$, J = 7.0 Hz), 4.29 (q, 2H, CH$_2$, J = 7.0 Hz), 6.26 (d, 1H, H-5, J = 2.7 Hz), 6.83 (dd, 1H, ar, J = 8.2, 2.2 Hz), 6.96 (d, 1H, ar, J = 2.2 Hz), 7.25 (s, 1H, H-1), 7.66 (d, 1H, ar, J = 8.2 Hz), 7.87 (d, 1H, NH, J = 2.7 Hz); IR 1700, 1740, 3380.

General Procedure To Prepare Ethyl 5,6-Dihydro-9-nitro-5-oxo-pyrazolo[1,5-*c*]quinazoline-2-carboxylates (3a and 4a). Compound **1a**[18]−**2a** (1.2 mmol) was added portionwise to cooled (0−5 °C) HNO$_3$ (90%, 4 mL). The reaction mixture was stirred at 0−5 °C until the disappearance of the starting material (TLC monitoring, eluting system AcOEt/cyclohexane 7:3). The solution was then poured onto ice (20 g) and the resulting solid was collected and washed with water. Compound **4a** displayed the following spectral data: ^{1}H NMR (DMSO-d_6) 1.36 (t, 3H, CH$_3$, J = 7.1 Hz), 4.40 (q, 2H, CH$_2$, J = 7.1 Hz), 7.51 (s, 1H, ar), 7.94 (s, 1H, H-1), 9.07 (s, 1H, ar), 12.58 (s, 1H, NH); IR 1680, 1780, 3240.

General Procedure To Prepare Ethyl 9-Amino-5,6-dihydro-5-oxo-pyrazolo[1,5-*c*]quinazoline-2-carboxylates (5a and 6a). Iron powder (1.3 g) was added to a solution of **3a**−**4a** (1.3 mmol) in glacial acetic acid (15 mL). The mixture was heated at 90 °C for 1 h and then evaporation at reduced pressure of the solvent yielded a solid residue that was dried and extracted in Soxhlet with acetone (500 mL). Evaporation at reduced pressure of the solvent afforded a solid that was purified by column chromatography [eluting system CHCl$_3$/MeOH (9.5:0.5)]. Compound **6a** displayed the following spectral data: ^{1}H NMR (DMSO-d_6) 1.34 (t, 3H, CH$_3$, J = 7.0 Hz), 4.37 (q, 2H, CH$_2$, J = 7.0 Hz), 5.43 (s, 2H, NH$_2$), 7.23 (s, 1H, ar), 7.37 (s, 1H, ar), 7.39 (s, 1H, H-1), 11.80 (br s, 1H, NH); IR 1750, 3300, 3520.

Ethyl 8-Chloro-5,6-dihydro-5-oxo-9-(1,2,4-triazol-4-yl)-pyrazolo[1,5-*c*]quinazoline-2-carboxylate (8a). Diformylhydrazine (4.5 mmol) and, drop by drop, trimethylsilyl chloride (22.5 mmol) and triethylamine (10.2 mmol) were successively added to a suspension of **6a** (1.5 mmol) in anhydrous pyridine (6 mL). The mixture was heated at 100 °C for 90 min, then evaporation at reduced pressure of the solvent yielded a solid that was treated with water (20 mL), collected, and washed with water: ^{1}H NMR (DMSO-d_6) 1.35 (t, 3H, CH$_3$, J = 7.0 Hz), 4.38 (q, 2H, CH$_2$, J = 7.0 Hz), 7.57 (s, 1H, ar), 7.75 (s, 1H, H-1), 8.61 (s, 1H, ar), 8.92 (s, 2H, triazole H-3 and H-5), 12.43 (br s, 1H, NH); IR 1750.

Ethyl 8-Chloro-5,6-dihydro-9-(3-formylpyrrol-1-yl)-5-oxo-pyrazolo[1,5-*c*]quinazoline-2-carboxylate (9a). A solution of 2,5-dimethoxy-3-tetrahydrofurancarboxaldehyde (0.94 mmol) in glacial acetic acid (3 mL) was added dropwise to a hot (90 °C) suspension of **6a** (0.62 mmol) in glacial acetic acid (10 mL). The mixture was heated at 90 °C for 10 min and then cooled and diluted with water (70 mL). The obtained solid was collected and purified by column chromatography [eluting system CHCl$_3$/MeOH (9:1)]: ^{1}H NMR (DMSO-d_6) 1.32 (t, 3H, CH$_3$, J = 7.3 Hz), 4.37 (q, 2H, CH$_2$, J = 7.3 Hz), 6.68 (s, 1H, ar), 7.19 (s, 1H, ar), 7.51 (s, 1H, ar), 7.80 (s, 1H, H-1), 7.94 (s, 1H, ar), 8.52 (s, 1H, ar), 9.79 (s, 1H, CHO), 12.34 (br s, 1H, NH).

General Procedure To Prepare 3-Hydroxy-5-(2-nitroaryl)-5*H*-furan-2-ones (26[25]**−28).** The title compounds were obtained from the suitable *o*-nitrobenzaldehyde (**23−25**)[23,24] and pyruvic acid, which serves also as a solvent. The synthesis was performed following the procedure described in ref 25 to prepare **26** with modifications. Dry hydrogen chloride was bubbled until saturation into a warmed (25−30 °C) mixture of the suitable *o*-nitrobenzaldehyde **23−25** (10 mmol) and pyruvic acid (21 mmol for the preparation of **26** and **27**, 50 mmol for the preparation of **28**). The mixture was left at room temperature until disappearance of the starting material [2−7 days, TLC monitoring, eluting system CHCl$_3$/MeOH (9:1)]. After addition of a small amount of petroleum ether and ethanol (1:1), the main product was collected. The residue was unstable upon recrystallization. Nevertheless, the crude product was pure enough and thus used without further purification.

26: ^{1}H NMR (DMSO-d_6) 6.46−6.50 (m, 2H, furan H-4 and H-5), 7.46 (d, 1H, ar, $J = 8.1$ Hz), 7.59−7.68 (m, 1H, ar), 7.75−7.82 (m, 1H, ar), 8.12 (dd, 1H, ar, $J = 8.1, 1.1$ Hz), 10.77 (br s, 1H, exchangeble with D_2O).

27: ^{1}H NMR (DMSO-d_6) 6.44−6.46 (m, 2H, furan H-4 and H-5), 7.48 (d, 1H, ar, $J = 8.6$ Hz), 7.85 (d, 1H, ar, $J = 8.6$ Hz), 8.21 (s, 1H, ar), 10.81 (br s, 1H, exchangeble with D_2O).

28: ^{1}H NMR (DMSO-d_6) 6.43−6.48 (m, 2H, furan H-4 and H-5), 7.69 (s, 1H, ar), 8.46 (s, 1H, ar), 10.86 (br s, 1H, exchangeble with D_2O).

General Procedure To Prepare Methyl 4-(2-Nitroaryl)-2-oxo-3-butenoates (29[25]−31). A suspension of furane **26**−**28** (8 mmol) in 30 mL of methanol, saturated with dry hydrogen chloride, was refluxed for 3−5 h [TLC monitoring, eluting system CHCl₃/MeOH (9:1)] and then set aside at room temperature for 12 h. The resulting solid was collected and washed with diethyl ether. Compound **31** displayed the following spectral data: ^{1}H NMR (DMSO-d_6) 3.85 (s, 3H, CH₃), 7.44 (d, 1H, butene H-3 or H-4, $J = 16.1$ Hz), 7.95 (d, 1H, butene H-4 or H-3, $J = 16.1$ Hz), 8.33 (s, 1H, ar), 8.46 (s, 1H, ar); IR 1700, 1740.

General Procedure To Prepare Methyl (±)-4,5-Dihydro-5-(2-nitroaryl)pyrazole-3-carboxylates (32−34). Anhydrous hydrazine (2.3 mmol) was added to a hot (90 °C) suspension of **29−31** (2.1 mmol) in absolute ethanol (5 mL). The resulting solution was refluxed for 30 min. The solid, which precipitated upon cooling, was collected and washed with diethyl ether. Compound **34** displayed the following spectral data: ^{1}H NMR (DMSO-d_6) 2.75−2.88 (m, 1H, pyrazoline H-4), 3.32−3.48 (m, 1H, pyrazoline H-4), 3.68 (s, 3H, CH₃), 5.29−5.34 (m, 1H, pyrazoline H-5), 7.73 (s, 1H, ar), 8.37 (s, 1H, ar), 8.75 (br s, 1H, NH); IR 1730, 3400.

General Procedure To Prepare Methyl (±)-4,5-Dihydro-5-(2-aminoaryl)pyrazole-3-carboxylates (35−37). PtO₂ [10% (w/w)] was added to a solution of **32** (3.2 mmol) in ethyl acetate (40 mL) or **33−34** (1.6 mmol) in ethanol (150 mL). The resulting mixture was hydrogenated in a Parr apparatus at 30 psi for 12 h. Elimination of the catalyst and evaporation at reduced pressure of the solvent afforded an oily residue that became solid upon treatment with a mixture of petroleum ether/diethyl ether (1:1). The residue was unstable upon recrystallization. Nevertheless, the crude product was pure enough and thus used without purification.

35: ^{1}H NMR (DMSO-d_6) 2.47−2.55 (m, 1H, pyrazoline H-4), 3.21−3.34 (m, 1H, pyrazoline H-4), 3.68 (s, 3H, CH₃), 4.95−4.99 (m, 3H, pyrazoline H-5 + NH₂), 6.52−6.66 (m, 2H, ar), 6.92−6.99 (m, 2H, ar), 8.68 (s, 1H, NH); IR 1720, 3320, 3430.

36: ^{1}H NMR (DMSO-d_6) 2.36−2.49 (m, 1H, pyrazoline H-4), 3.23−3.35 (m, 1H, pyrazoline H-4), 3.67 (s, 3H, CH₃), 4.89−5.01 (m, 1H, pyrazoline H-5), 5.32 (br s, 2H, NH₂), 6.53 (d, 1H, ar, $J = 8.2$ Hz), 6.67 (s, 1H, ar), 6.97 (d, 1H, ar, $J = 8.2$ Hz), 8.70 (s, 1H, NH).

37: ^{1}H NMR (DMSO-d_6) 2.36−2.50 (m, 1H, pyrazoline H-4), 3.12−3.26 (m, 1H, pyrazoline H-4), 3.67 (s, 3H, CH₃), 4.89−5.03 (m, 1H, pyrazoline H-5), 5.51 (s, 2H, NH₂), 6.82 (s, 1H, ar), 7.19 (s, 1H, ar), 8.77 (s, 1H, NH).

General Procedure To Prepare Methyl (±)-1,5,6,10b-Tetrahydro-5-oxo-pyrazolo[1,5-c]quinazoline-2-carboxylates (12a−14a). The title compounds were prepared by reacting **35−37** (2.7 mmol) with triphosgene (1.1 mmol) and triethylamine (6.6 mmol), as described above for the preparation of **2a**. Compound **14a** displayed the following spectral data: ^{1}H NMR (DMSO-d_6) 3.13−3.29 (m, 1H, H-1), 3.57−3.72 (m, 1H, H-1), 3.80 (s, 3H, CH₃), 5.23−5.36 (m, 1H, H-10b), 7.12 (s, 1H, ar), 7.56 (s, 1H, ar), 10.16 (s, 1H, NH); IR 1740.

Methyl 8,9-Dichloro-5,6-dihydro-5-oxo-pyrazolo[1,5-c]quinazoline-2-carboxylate (7a). A solution of tetrachloro-1,2-benzoquinone (0.54 mmol) in anhydrous toluene (4 mL) was added dropwise to a hot (120 °C) suspension of **14a** (0.54 mmol) in anhydrous toluene (10 mL). The mixture was heated at 120 °C for 1 h and then cooled at room temperature. The resulting solid was collected and washed with diethyl ether: ^{1}H NMR (DMSO-d_6) 3.91 (s, 3H, CH₃), 7.50 (s, 1H, ar), 7.84 (s, 1H, H-1), 8.56 (s, 1H, ar).

General Procedure To Prepare 5,6-Dihydro-5-oxo-pyrazolo[1,5-c]quinazoline-2-carboxylic Acids (2b−4b, 8b). A suspension of **2a−4a**, **8a** (1.5 mmol) in glacial acetic acid (6 mL) and 6 N HCl (1.2 mL) was heated at 100 °C until disappearance of the starting material [TLC monitoring, eluting system CHCl₃/MeOH/AcOH (8:1.5:0.5)]. The mixture was cooled and then the solid was collected and washed with water. Compound **2b** and **8b** displayed the following spectral data.

2b: ^{1}H NMR (DMSO-d_6) 7.35−7.40 (m, 2H, ar), 7.66 (s, 1H, H-1), 8.17 (d, 1H, ar, $J = 8.5$ Hz), 12.14 (s, 1H, exchangeble with D_2O), 13.34 (s, 1H, exchangeble with D_2O); IR 1680, 1760, 3400.

8b: ^{1}H NMR (DMSO-d_6) 7.56 (s, 1H, ar), 7.67 (s, 1H, H-1), 8.58 (s, 1H, ar), 8.91 (s, 2H, triazole H-3 and H-5) 12.4 (br s, 1H, exchangeble with D_2O); IR 1680, 1740, 3450.

8,9-Dichloro-5,6-Dihydro-5-oxo-pyrazolo[1,5-c]quinazoline-2-carboxylic Acid (7b). A solution of KOH (1.5 N, 12 mL) was added to a suspension of **7a** (1.5 mmol) in methanol (12 mL). The reaction mixture was heated at 100 °C for 15 min and then cooled. The resulting solid residue was filtered and dissolved in the minimum amount of water. The solution was acidified with glacial acetic acid to afford a solid that was collected and washed with water: ^{1}H NMR (DMSO-d_6) 7.49 (s, 1H, ar), 7.75 (s, 1H, H-1), 8.35 (s, 1H, ar), 12.21 (s, 1H, exchangeble with D_2O), 13.43 (br s, 1H, exchangeble with D_2O).

8-Chloro-5,6-dihydro-9-(3-formylpyrrol-1-yl)-5-oxo-pyrazolo[1,5-c]quinazoline-2-carboxylic Acid (9b). A solution of KOH (1.5 N, 12 mL) was added to a suspension of **9a** (1.5 mmol) in methanol (12 mL). The reaction mixture was heated at 100 °C for 15 min and then cooled and acidified with 6 N HCl to yield a solid that was collected and washed with water: ^{1}H NMR (DMSO-d_6) 6.69 (s, 1H, ar), 7.20 (s, 1H, ar), 7.53 (s, 1H, ar), 7.82 (s, 1H, H-1), 7.95 (s, 1H, ar), 8.51 (s, 1H, ar), 9.80 (s, 1H, CHO), 12.36 (br s, 1H, exchangeble with D_2O).

General Procedure To Prepare (±)-1,5,6,10b-Tetrahydro-5-oxo-pyrazolo[1,5-c]quinazoline-2-carboxylic Acids (12b−14b). The title compounds were obtained from **12a−14a** (1.4 mmol), by following the procedure described above for the preparation of **2b−4b** and **8b**. Compound **14b** displayed the following spectral data: ^{1}H NMR (DMSO-d_6) 3.11−3.27 (m, 1H, H-1), 3.58−3.67 (m, 1H, H-1), 5.20−5.32 (m, 1H, H-10b), 7.11 (s, 1H, ar), 7.53 (s, 1H, ar), 10.10 (s, 1H, exchangeble with D_2O), 13.40 (br s, 1H, exchangeble with D_2O); IR 1680, 1740, 3200.

General Procedure To Prepare 5-(2-Aminoaryl)pyrazole-3-carboxylic Acids (38 and 39). A suspension of **21−22** (0.90 mmol) in 3 N NaOH (3 mL) was heated at 100 °C for 30 min. Dilution with water (10 mL) and acidification of the clear solution with glacial acetic acid afforded a solid that was collected and washed with water. Compound **39** displayed the following spectral data: ^{1}H NMR (DMSO-d_6) 6.53−6.78 (m, 4H, 2ar + NH₂), 7.14 (s, 1H, ar), 7.53 (d, 1H, ar, $J = 8.4$ Hz); IR 1680, 3240, 3380, 3480.

General Procedure To Prepare (±)-5,6-Dihydro-pyrazolo[1,5-c]quinazoline-2,5-dicarboxylic Acids (10b and 11b). The title compounds were prepared by reacting **38−39** (1 mmol), with an excess of glyoxylic acid monohydrate (1.5 mmol), as described above for the preparation of **10a** and **11a**. Compound **11b** displayed the following spectral data: ^{1}H NMR (DMSO-d_6) 5.95 (s, 1H, H-5), 6.77 (d, 1H, ar, $J = 8.2$ Hz), 6.92 (s, 1H, ar), 7.12 (s, 1H, H-1), 7.58 (d, 1H, ar, $J = 8.2$ Hz), 7.68 (s, 1H, NH); IR 1700, 3250, 3500.

Pharmacology

Binding Assay. Rat cortical synaptic membrane preparation, [³H]glycine, [³H]AMPA, and [³H]-(+)-MK-801 binding experiments were performed following the procedures described in refs 11, 31, and 16, respectively.

High Affinity [³H]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; specific activity, 58 Ci/mmol) and tested compounds in a total 0.5 mL volume. 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 of ice-cold buffer.

Electrophysiological Assay. The cortical wedge preparation described by Mannaioni et al. [32] 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 (component, mM: NaCl, 135; CaCl$_2$, 2.4; KH$_2$PO$_4$, 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 (5μM) and NMDA (5μM) 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 antagonist-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.

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 to four displacement curves on the basis of four to six scalar concentrations of the test compound in triplicate using the ALLFIT computer program[33] and, in the case of tritiated glycine and AMPA binding, converted to K_i values by application of the Cheng−Prusoff equation.[34] 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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