Brief Articles

4,5-Dihydro-1,2,4-triazolo[1,5-*a*]quinoxalin-4-ones: Excitatory Amino Acid Antagonists with Combined Glycine/NMDA and AMPA Receptor Affinity

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A series of 4,5-dihydro-1,2,4-triazolo[1,5-*a*]quinoxalin-4-ones bearing different substituents on the benzo-fused ring and at position 2 were synthesized and biologically evaluated for their binding at glycine/NMDA and AMPA receptors. Most of the reported compounds show combined glycine/NMDA and AMPA receptor binding activity providing further evidences of the structural similarities of the binding pockets of both receptor recognition sites. Moreover, this study has pointed out some differences for the binding at each receptor type. In particular, for the glycine/NMDA receptor−ligand interaction, the presence of a free acidic function at position 2 and an electron-withdrawing substituent(s) nonbulkier than chlorine atom(s) on the benzo-fused moiety is required. Functional antagonism at the NMDA receptor−ion channel complex was also performed on some selected compounds.

Introduction

Glutamate receptors (GluRs) mediate most of the excitatory neurotransmission in the mammalian central nervous system. GluRs have been classified into two general classes of receptors: those that form ion channels or "ionotropic", and those that are linked to G-protein or "metabotropic".[1,2] The ionotropic GluRs are further subdivided according to their preferential agonists as NMDA (*N*-methyl-D-aspartate), AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid), and KA (kainic acid).[3] Like other ligand-gated ion channels, the NMDA receptor is modulated by a number of different molecules acting at different sites, and among them the amino acid glycine, binding at the allosteric strycnine-insensitive glycine site (glycine/NMDA), is particularly important for its synergic action with glutamate for the NMDA receptor activation.[4]

Overstimulation of both NMDA and AMPA receptors by excessive endogenous glutamate, which occurs in several pathological conditions, can cause excess excitation that initiates neuronal cell death. Thus, antagonists at both NMDA and AMPA receptors are attractive therapeutic targets in many neurological diseases, such as focal and global ischemia[5,6] and seizure disorders,[7−9] and in the protection of neuronal degeneration in the retina.[10] It has been established that there is a considerable similarity in structural requirements for binding at the glycine/NMDA and AMPA recognition sites.[9,11−12] In fact, structure−activity relationships (SAR) on quinoxaline-2,3-dione derivatives, which have been reported to have glycine/NMDA and AMPA receptor affinity, have led to the identification of the common structural requirements for their anchoring to the recognition site of both receptors.[9,12−17]

Thus, the purpose of this study was to prepare some 1,2,4-triazolo[1,5-*a*]quinoxalin-4-ones with combined glycine/NMDA and AMPA receptor antagonist activities. In these new tricyclic quinoxalin-4-ones the nitrogen atom at position 3 is replacing the 3-carbonyl oxygen of the bicyclic quinoxalinediones. Different substituents have been placed on the benzo-fused ring and at position 2 of the tricyclic system to assess the similarities and/or differences for the binding at each receptor in these new kinds of ligands.

Chemistry

The synthesis of the 4,5-dihydro-1,2,4-triazolo[1,5-*a*]-quinoxaline derivatives **1a**−**i, 2a**−**g,i**−**l,** and **3**−**21** is illustrated in Schemes 1 and 2. The triazoloquinoxaline-2-carboxylic esters **1a**−**f,h** were prepared through the intermediates **22**−**25a**−**g**, following reported procedures[18,19] (Scheme 1). The tricyclic 7-nitro and 7-bromo esters **1g,i** were prepared from the diazonium salt of the 7-amino ester **1h** with sodium nitrite or copper(II) bromide, respectively. The hydrolysis of the esters **1a**−**g,i** gave the corresponding acids **2a**−**g,i**, while the nitro acids **2j**−**l** ensued by nitration of the acids **2a**−**c**.

By reacting the tricyclic 7-chloro ester **1b** with ethyl iodide (Scheme 2), a mixture of 4-ethoxy ester **3** and 5-*N*-ethyl ester **4** was obtained. After chromatographic separation, the main product of the alkylation reaction, i.e., the *N*-ethyl ester **4**, was hydrolyzed to the corresponding acid **5**. In addition, reduction of the tricyclic

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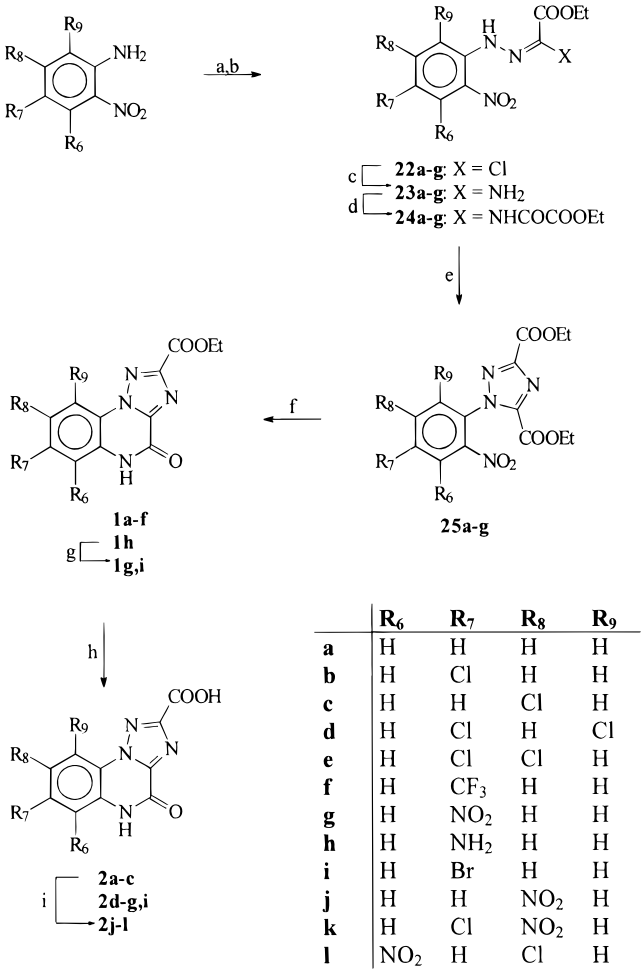
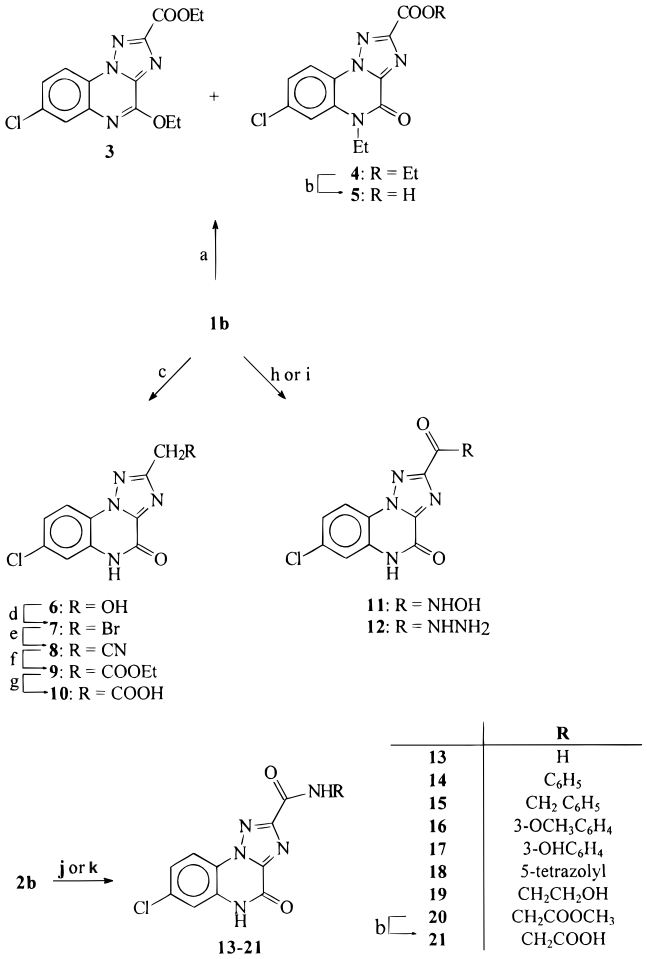
Scheme 1[a]



	R_6	R_7	R_8	R_9
a	H	H	H	H
b	H	Cl	H	H
c	H	H	Cl	H
d	H	Cl	H	Cl
e	H	Cl	Cl	H
f	H	CF_3	H	H
g	H	NO_2	H	H
h	H	NH_2	H	H
i	H	Br	H	H
j	H	H	NO_2	H
k	H	Cl	NO_2	H
l	NO_2	H	Cl	H

[a] (a) Diazotization; (b) $CH_3COCHClCOOEt$; (c) NH_3(g); (d) ClCOCOOEt; (e) heating above mp or concd H_2SO_4; (f) Fe/CH_3COOH; (g) diazotization, $NaNO_2$ or $CuBr_2$; (h) NaOH, HCl; (i) 90% HNO_3.

Scheme 2[a]



	R
13	H
14	C_6H_5
15	$CH_2C_6H_5$
16	3-$OCH_3C_6H_4$
17	3-OHC_6H_4
18	5-tetrazolyl
19	CH_2CH_2OH
20	CH_2COOCH_3
21	CH_2COOH

[a] (a) EtI; (b) NaOH, HCl; (c) $LiBH_4$; (d) PBr_3; (e) NaCN; (f) concd H_2SO_4/EtOH; (g) NaOH, CH_3COOH; (h) NH_2OH·HCl/NaOH; (i) N_2H_4·H_2O; (j) $SOCl_2$, NH_4OH; (k) RNH_2.

7-chloro ester **1b** yielded the alcohol **6**, which was reacted with phosphorus tribromide to give the bromomethyl derivative **7**. By reacting **7** with sodium cyanide the acetonitrile **8** was obtained. The latter was transformed into the ethyl acetate **9**, which was hydrolyzed to give the corresponding 2-acetic acid **10**. Finally, by reacting **1b** with hydroxylamine hydrochloride or hydrazine hydrate, the 2-hydroxamic acid **11** and 2-hydrazide **12** were obtained, respectively.

The preparation of the 2-carboxamide derivatives **13**−**20** from the 7-chloro acid **2b** is also illustrated in Scheme 2. The glycine methyl ester **20** was hydrolyzed to the corresponding acid **21**.

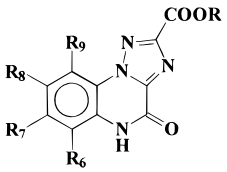
Results and Discussion

The triazoloquinoxalines **1a**−**f**,**h**−**i**, **2a**−**g**,**i**−**l**, **5**,**6**, and **8**−**21** were tested for their ability to displace both tritiated glycine and AMPA from their specific binding in rat cortical membranes. The binding data are shown in Tables 1 and 2. The results listed in Table 1 show that all the acids **2** are more active than the corresponding esters **1** especially at the level of the glycine/NMDA receptor. The enhanced glycine/NMDA affinity of the acids **2** with respect to the corresponding esters **1** arises from a strong interaction between the anionic carboxylate residue at position 2 and a cationic proton donor site of the receptor.[20]

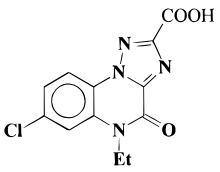
The importance of the presence of the NH proton donor and of electron-withdrawing substituent(s) on the benzo-fused moiety is illustrated by the inactivity of the 5-*N*-ethyl acid **5** and 7-amino ester **1h**, respectively, which are inactive at both receptors. However, the position and the nature of the substituent on the benzo-fused moiety are very important. A chlorine atom at position 7 enhances receptor affinity (see **2b** vs **2a**), while the same halogen at position 8 decreases it (see **2c** vs **2b**) at both receptors. The same applies to the 7-nitro acid **2g** and 8-nitro acid **2j**. However, the replacement of the 7-chlorine atom of **2b** with a bulkier substituent such as a trifluoromethyl or nitro group or a bromine atom (compounds **2f**,**g**,**i**, respectively) decreases glycine/NMDA affinity, while it has little or no effect on AMPA binding activity, with the exception of the 7-bromo derivative **2i** which, in agreement with the literature data,[9,16] is 3-fold less active than **2b**. It follows that in the glycine/NMDA receptor there is a size-limited region which cannot well-accommodate a 7- or 8-substituent bulkier than a chlorine atom.

The disubstitution of the benzo-fused moiety has different effects on the binding activity depending on the receptor type. In fact, the 7,9-dichloro acid **2d** and 7,8-dichloro acid **2e** are respectively equally active or

Table 1. Binding Activities at Glycine/NMDA and AMPA Receptors of Tricyclic Esters and Acids



1a-f, h-i, 2a-g, i-l

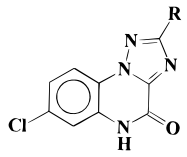


5

						K_i[a] (μM)	
compd	R	R_6	R_7	R_8	R_9	[^{3}H]glycine	[^{3}H]AMPA
1a	Et	H	H	H	H	16.7 ± 1.2	28.5 ± 1.2
1b	Et	H	Cl	H	H	3.0 ± 0.4	2.6 ± 0.5
1c	Et	H	H	Cl	H	88.2 ± 15.0	72.1 ± 19.5
1d	Et	H	Cl	H	Cl	4.8 ± 0.5	4.9 ± 1.4
1e	Et	H	Cl	Cl	H	0.71 ± 0.08	33.9 ± 5.9
1f	Et	H	CF_3	H	H	1.8 ± 0.3	3.6 ± 0.4
1h	Et	H	NH_2	H	H	>100	>100
1i	Et	H	Br	H	H	2.6 ± 0.2	3.3 ± 0.7
2a	H	H	H	H	H	1.1 ± 0.02	13.6 ± 0.8
2b	H	H	Cl	H	H	0.17 ± 0.02	0.78 ± 0.15
2c	H	H	H	Cl	H	3.5 ± 0.8	18.4 ± 3.4
2d	H	H	Cl	H	Cl	0.14 ± 0.02	1.53 ± 0.2
2e	H	H	Cl	Cl	H	0.074 ± 0.003	1.3 ± 0.2
2f	H	H	CF_3	H	H	0.57 ± 0.08	0.90 ± 0.09
2g	H	H	NO_2	H	H	0.71 ± 0.04	0.60 ± 0.08
2i	H	H	Br	H	H	0.39 ± 0.09	4.4 ± 0.7
2j	H	H	H	NO_2	H	79.4 ± 8.8	10.7 ± 0.7
2k	H	H	Cl	NO_2	H	2.2 ± 0.3	1.2 ± 0.08
2l	H	NO_2	H	Cl	H	4.5 ± 0.5	4.0 ± 0.5
5						88.0 ± 11.5	>100

[a] K_i values are means ± SEM of 3−4 separate determinations in triplicate. The tested compounds were dissolved in DMSO.

Table 2. Binding Activity at Glycine/NMDA and AMPA Receptors of Compounds **6** and **8**−**21**



		K_i[a] (μM)	
compd	R	[^{3}H]glycine	[^{3}H]AMPA
6	CH_2OH	7.5 ± 0.9	4.4 ± 0.8
8	CH_2CN	9.7 ± 1.8	2.0 ± 0.2
9	CH_2COOEt	6.5 ± 1.8	2.1 ± 0.4
10	CH_2COOH	1.1 ± 0.04	3.7 ± 0.5
11	CONHOH	1.1 ± 0.09	3.0 ± 0.4
12	$CONHNH_2$	9.6 ± 0.5	5.0 ± 0.5
13	$CONH_2$	7.2 ± 1.4	11.7 ± 1.7
14	$CONHC_6H_5$	NT[b]	NT[b]
15	$CONHCH_2C_6H_5$	NT[b]	NT[b]
16	CONH-3-$OCH_3C_6H_4$	NT[b]	NT[b]
17	CONH-3-OHC_6H_4	NT[b]	NT[b]
18	CONH-5-tetrazolyl	5.1 ± 0.3	2.4 ± 0.2
19	$CONHCH_2CH_2OH$	27.7 ± 5.9	7.7 ± 1.9
20	$CONHCH_2COOCH_3$	5.4 ± 0.7	4.1 ± 0.4
21	$CONHCH_2COOH$	8.8 ± 0.9	3.2 ± 0.4

[a] See Table 1. [b] Due to the insolubility of the compound in the medium binding assay, testing was not possible.

more active at the glycine/NMDA receptor and less active at the AMPA receptor than the 7-monochloro acid **2b**. In the case of glycine/NMDA receptor, the size-limited tolerance observed in the monosubstitution of the benzo-fused moiety is confirmed by the binding results of the 7,8-disubstituted derivatives (see **2k** vs **2e**). On the contrary, the 7-chloro-8-nitro acid **2k** and 7,8-dichloro acid **2e** have similar affinities at the AMPA receptor, demonstrating that in this receptor there is a larger lipophilic pocket able to accommodate bulky substituent(s). As expected, the 6-nitro-8-chloro acid **2l** displays low affinity at both receptors.

Table 3. Functional Antagonism at Glycine/NMDA Site of Some Selected Triazoloquinoxalin-4-ones

compd	[^{3}H]MK-801[a] EC_{50} (μM)
1a	69.0 ± 12.0
1b	22.1 ± 1.5
1e	1.5 ± 0.28
1h	>100
2a	4.9 ± 0.9
2b	2.9 ± 0.3
2c	32.5 ± 1.9
2d	1.35 ± 1.2
2e	0.30 ± 0.05
2f	3.18 ± 0.21
2g	7.8 ± 0.7
2i	3.7 ± 0.24
2j	>100
2l	>100
5	>100
5,7-dichlorokynurenic acid	0.33 ± 0.05

[a] Concentrations giving 50% inhibition of stimulated [^{3}H]-(+)-MK-801 binding. All assays were carried out in the presence of 10 μM glutamate: 100% binding = glutamate + buffer; 0% binding = glutamate + phencyclidine hydrochloride (100 μM). The results were calculated from 3−4 separate determinations in triplicate.

The importance in the triazoloquinoxalines of a free acidic group at position 2 is further confirmed by the introduction of different substituents in this position of the parent acid **2b.** The binding results shown in Table 2 indicate that, in general, the replacement of the 2-carboxylic group of **2b** results in a reduction in binding activity at both receptors. Moreover, the decreased binding activities of the 2-acetic acid **10** and hydroxamic acid **11** suggest that the spatial position of the free acidic function is critical for receptor−ligand interaction at both receptors. Moving the acidic function in the side chain at position 2 further, as in compounds **18** and **21**, produces a progressive reduction in binding activity at glycine/NMDA receptor. The amides **14**−**17**, prepared to investigate the influence of a lipophilic moiety on the side chain at position 2, unfortunately could not be tested because of their insolubility in the medium of the binding assay.

Functional antagonism at the NMDA receptor−ion channel complex was demonstrated by the ability of some triazoloquinoxalines to inhibit the binding of the channel-blocking agent [^{3}H]-(+)-MK-801[12,21−22] in membranes incubated with 10 μM glutamate and in the nominal absence of glycine (Table 3). The results shown in Table 3 indicate that, as in the case of [^{3}H]glycine displacement assays, compound **2e** is the most potent compound tested with an EC_{50} of 0.30 ± 0.05 μM. This value is very close to that obtained in our experiments with the glycine/NMDA antagonist 5,7-dichlorokynurenic acid (0.33 ± 0.05 μM). The EC_{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. Figure 1 shows that the inhibition of [^{3}H]-(+)-MK-801 binding to the channel of activated NMDA receptor by **2e** can be completely reversed by increasing glycine concentrations, indicating competition between the two compounds for the regulatory site on the receptor.

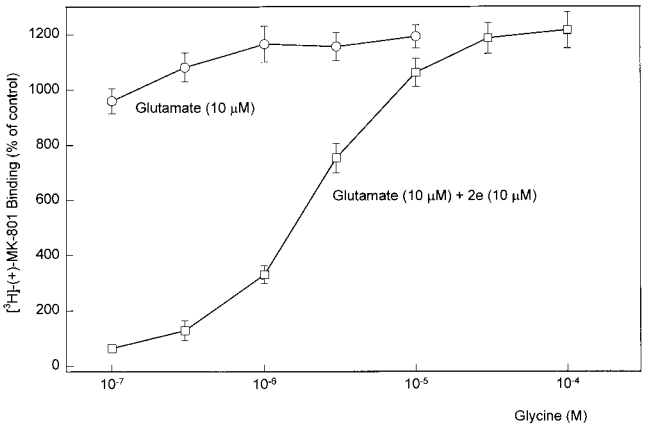


Figure 1. Effect of increasing glycine concentrations on the antagonist action of **2e** on NMDA receptor-mediated [^{3}H]-(+)-MK-801 binding to rat cortical membranes. Ordinate values represent percentages of specific [^{3}H]-(+)-MK-801 binding in the nominal absence of glycine and glutamate. In the absence of glycine, the specific [^{3}H]-(+)-MK-801 binding was equal to 568% or 41% of controls in the presence of 10 μM glutamate plus buffer or 10 μM glutamate plus 10 μM **2e**, respectively. Points on the graph represent the means ± SEM from three experiments in triplicate.

In conclusion, the synthesis of these triazoloquinoxalines has produced some nonselective antagonists of the glycine/NMDA and AMPA receptors. This result provides further evidence of the structural similarities of the binding pockets of both receptor recognition sites and confirms our original hypothesis that the 3-carbonyl oxygen of the bicyclic quinoxalinediones can be replaced by the nitrogen atom at position 3 of the triazoloquinoxalines. Moreover, the SAR on these new kinds of glycine/NMDA and AMPA receptor ligands have pointed out some differences for the binding at each receptor type. In particular, for the glycine/NMDA receptor−ligand interaction, the presence of a free acidic function at position 2 and substituent(s) on the benzo-fused moiety nonbulkier than chlorine atom(s) is required.

It follows that the triazoloquinoxalin-4-one tricyclic system may represent a structure which, suitably modified, could lead to selective glycine/NMDA or AMPA receptor ligands. Further modifications of these molecules to improve biological activity and/or 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 data of the newly synthesized compounds are listed in Tables 4 and 5.

Ethyl N^1-(2-Nitroaryl)hydrazono-N^2-chloroacetates 22a−g. All the title compounds were prepared by reacting the diazonium salt of the suitable 2-nitroaniline with ethyl 2-chloro-3-oxobutanoate. Compounds **22a−c** were prepared from the 2-nitroaryldiazonium chloride.[18,23] Compounds **22d−g** were obtained by reacting at room temperature equimolar amount (40 mmol) of the suitable 2-nitroaryldiazonium tetrafluoborate and ethyl 2-chloro-3-oxobutanoate in a small amount of MeOH. The synthesis of 4,6-dichloro-2-nitrophenyl- and 2,4-dinitrophenyldiazonium tetrafluoborate is reported in refs 24 and 25, respectively. The unknown 4,5-dichloro-2-nitrophenyl- and 4-(trifluoromethyl)-2-nitrophenyldiazonium tetrafluoborates were obtained following the method described in ref 24. Compound **22b** displays the following spectral data: ^{1}H NMR ($CDCl_3$) 1.44 (t, 3H, CH_3), 4.43 (q, 2H, CH_2), 7.60 (dd, 1H, ar, J = 9.1, 2.3 Hz), 7.94 (d, 1H, ar, J = 9.1 Hz), 8.24 (d, 1H, ar, J = 2.3 Hz), 11.33 (s, 1H, NH); IR 1750, 3100, 3120, 3300.

Table 4. Physical Data of the Final Tricyclic Compounds

compd	mp (°C)	cryst solv	% yield
1b	280−282	acetone	78
1d	263−264	acetone	70
1e	298−300	EtOH	82
1f	>300	EtOH	86
1g	280−281	acetone	45
1h	>300	acetone	92
1i	284−285	acetonitrile	18
2b	>300	H_2O	93
2c	264−266 dec	EtOH/H_2O	94
2d	>300	EtOH	83
2e	>300	EtOH	84
2f	>300	EtOH	98
2g	>300	EtOH	82
2i	>300	EtOH	90
2j	237−240	EtOH	67
2k	>300	EtOH	68
2l	>300	EtOH	63
3	>300[a]		10
4	>300[a]		40
5	>300	EtOH	95
6	265−267	EtOH/DMF	98
7	>300	EtOH	65
8	279−280	acetone	30
9	200−202	EtOH	49
10	254−255	EtOH	88
11	>300	EtOH	87
12	>300	DMF/H_2O	98
13	>300	DMF/H_2O	90
14	>300	DMF/H_2O	90
15	>300	DMF/H_2O	90
16	>300	DMF/H_2O	71
17	>300	DMF/H_2O	89
18	>300	DMF	57
19	298−300 dec	DMF/H_2O	62
20	>300	DMF	85
21	>300	DMF/H_2O	81

[a] Compound purified by column chromatography, eluting system ethyl acetate/cyclohexane (1:1).

Table 5. Physical Data of the Intermediate Compounds

compd	mp (°C)	cryst solv	% yield
22d	84−85	EtOH	20
22e	125−126	EtOH	57
22f	95−96	EtOH	68
22g	120−121	EtOH	76
23d	97−98	Et_2O/petroleum ether	98
23e	179−180	EtOH	86
23f	108−109	Et_2O	87
23g	108−109	EtOH	76
24b	158−159	MeOH	74
24d	126−127	MeOH	54
24e	167−168	EtOH	86
24f	167−169	Et_2O	55
24g	152−155	EtOH	77
25b	165−166	EtOH	63
25d	149−150	EtOH	95
25e	163−165	EtOH	87
25f	141−142	EtOH	41
25g	164−165	EtOH	69

Ethyl N^1-(2-Nitroaryl)-N^2-oxamidrazonates 23a−g. The title compounds were obtained from **22a−g** (49 mmol) as described for the synthesis of **23a−b.**[18,23] Compound **23b** displays the following spectral data: ^{1}H NMR ($CDCl_3$) 1.43 (t, 3H, CH_3), 4.42 (q, 2H, CH_2), 4.97 (br s, 2H, NH_2), 7.51 (dd, 1H, ar, J = 9.2, 2.4 Hz), 7.87 (d, 1H, ar, J = 9.2 Hz), 8.17 (d, 1H, ar, J = 2.4 Hz), 10.04 (br s, 1H, NH); IR 1730, 3320, 3340, 3430.

Ethyl N^1-(2-Nitroaryl)-N^3-ethoxalyl-N^2-oxamidrazonates 24a−g. The title compounds were obtained from **23a−g** (2.18 mmol) and ethyloxalyl chloride (4.4 mmol) as described for the synthesis of **24a,c**.[18,19] Compound **24b** displays the following spectral data: ^{1}H NMR ($CDCl_3$) 1.44 (t, 3H, CH_3), 1.49 (t, 3H, CH_3), 4.43 (q, 2H, CH_2), 4.54 (q, 2H, CH_2), 7.53 (dd, 1H, ar, J = 9.2, 2.3 Hz), 8.76 (d, 1H, ar, J = 9.2 Hz), 8.20 (d, 1H, ar, J = 2.3 Hz), 9.30 (br s, 1H, NH), 13.27 (br s, 1H, NH); IR 1720, 1730, 1775, 3100, 3280, 3400, 3420.

Diethyl 1-(2-Nitroaryl)-1,2,4-triazole-3,5-dicarboxylates 25a−g. Method A. Compounds **25a−c,e−g** were obtained from **24a−c,e−g** (5.2 mmol) as described for the synthesis of **25a,c.**[18−19]

Method B. Concentrated H_2SO_4 (12 mL) was added dropwise and under stirring to finely crushed **24d** (2 mmol). The solution was stirred at room temperature for 1 h and then poured onto crushed ice (80 g). The resulting suspension was extracted with ethyl acetate (50 mL × 3). The organic layers were washed with a diluted solution of $NaHCO_3$ (1%, 80 mL × 3) and H_2O (100 mL × 2) and then dried (Na_2SO_4). Evaporation at reduced pressure of the solvent yielded solid crude **25d**. Compound **25b** displays the following spectral data: ^{1}H NMR ($CDCl_3$) 1.36 (t, 3H, CH_3), 1.46 (t, 3H, CH_3), 4.37 (q, 2H, CH_2), 4.54 (q, 2H, CH_2), 7.41 (d, 1H, ar, J = 8.4 Hz), 7.80 (dd, 1H, ar, J = 8.4, 1.6 Hz), 8.30 (d, 1H, ar, J = 1.6 Hz); IR 1735, 1755.

Ethyl 4,5-Dihydro-4-oxo-1,2,4-triazolo[1,5-*a*]quinoxaline-2-carboxylates 1a−f,h. The title compounds were obtained from **25a−g** (5.2 mmol) as described for the synthesis of **1a,c**.[18,19] Compound **1b** displays the following spectral data: ^{1}H NMR (DMSO-d_6) 1.39 (t, 3H, CH_3), 4.45 (q, 2H, CH_2), 7.42−7.46 (m, 2H, ar), 8.13 (d, 1H, ar, J = 8.4 Hz), 12.54 (br s, 1H, NH); IR 1705, 1750.

4,5-Dihydro-4-oxo-1,2,4-triazolo[1,5-*a*]quinoxaline-2-carboxylic Acids 2a−f. The title compounds were obtained by hydrolysis of **1a−f** (2.4 mmol) as described for the synthesis of **2a**.[19] Compound **2b** displays the following spectral data: ^{1}H NMR (DMSO-d_6) 7.48−7.42 (m, 2H, ar), 8.13 (d, 1H, ar, J = 8.4 Hz), 12.54 (s, 1H, NH); IR 1695, 1725.

Ethyl 4,5-Dihydro-7-nitro-4-oxo-1,2,4-triazolo[1,5-*a*]quinoxaline-2-carboxylate (1g). Compound **1h** (1.1 mmol) was carefully added to a frozen (−5 °C) solution of nitrosylsulfuric acid (1.1 mmol) in concentrated H_2SO_4 (1 mL). The mixture was stirred at 0 °C for 2 h and then at 10 °C for 1 h and finally poured onto crushed ice (15 g). The solid was quickly filtered off, and the solution was cooled (0 °C) and treated dropwise with a concentrated solution of $NaBF_4$ (5.9 mmol in 2 mL of H_2O). The mixture was stirred at 0−5 °C for 1.5 h. The resulting solid was collected and washed with a diluted solution (25%) of $NaBF_4$ and then with cold MeOH. This 7-aryldiazonium tetrafluoborate was carefully added to a stirred and warm (30 °C) solution of $NaNO_2$ (10%, 55 mL). The mixture was stirred at room temperature overnight and then extracted with ethyl acetate (50 mL × 3). Evaporation of the dried (Na_2SO_4) organic solvent yielded the crude title compound: ^{1}H NMR (DMSO-d_6) 1.40 (t, 3H, CH_3), 4.47 (q, 2H, CH_2), 8.18−8.39 (m, 3H, ar), 12.78 (br s, 1H, NH); IR 1695, 1735.

Ethyl 7-Bromo-4,5-dihydro-4-oxo-1,2,4-triazolo[1,5-*a*]quinoxaline-2-carboxylate (1i). Compound **1h** (1.83 mmol) was carefully added to a cooled (0−5 °C) mixture of $CuBr_2$ (2.29 mmol) and *tert*-butyl nitrite (2.79 mmol). The mixture was stirred at room temperature overnight and then poured onto crushed ice (20 g) and bleached with HCl (6 N). The resulting solid was collected, washed with H_2O, and purified by column chromatography, eluting system $CHCl_3$/acetonitrile (8.5:1.5): ^{1}H NMR (DMSO-d_6) 1.38 (t, 3H, CH_3), 4.45 (q, 2H, CH_2), 7.57−7.62 ((m, 2H, ar), 8.09 (d, 1H, ar, J = 8.7 Hz), 12.56 (br s, 1H, NH); IR 1690, 1740.

4,5-Dihydro-4-oxo-1,2,4-triazolo[1,5-*a*]quinoxaline-2-carboxylic Acids 2g,i. The title compounds were obtained from the esters **1g,i** (2.4 mmol) as described for the synthesis of **2a**.[19] Compound **2g** displays the following spectral data: ^{1}H NMR (DMSO-d_6) 8.23−8.35 (m, 3H, ar), 12.77 (br s, 1H, NH); IR 1720.

General Procedure to Prepare Tricyclic Nitroaryl Acids 2j−l. Compounds **2a−c** (0.6 mmol) were carefully 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 crushed ice (20 g). The resulting solid was collected and washed with H_2O. Compound **2k** displays the following spectral data: ^{1}H NMR (DMSO-d_6) 7.65 (s, 1H, ar), 8.79 (s, 1H, ar), 12.9 (br s, 1H, NH); IR 1730.

Ethyl 7-Chloro-4-ethoxy-1,2,4-triazolo[1,5-*a*]quinoxaline-2-carboxylate (3) and Ethyl 7-Chloro-5-*N*-ethyl-4,5-dihydro-4-oxo-1,2,4-triazolo[1,5-*a*]quinoxaline-2-carboxylate (4). Ethyl iodide (4.29 mmol) and NaH (8.3 mmol) were successively added to a stirred solution of **1b** (3.1 mmol) in anhydrous dimethylformamide (DMF) (35 mL). The mixture was stirred at room temperature for 6 h and then poured onto crushed ice (40 g) to give a mixture of compounds **3** and **4**. The mixture was collected, washed with H_2O, and separated by column chromatography, eluting system cyclohexane/ethyl acetate (1:1). Compound **3** was obtained by evaporation at reduced pressure of the first running eluates, while compound **4** was obtained from evaporation at reduced pressure of the second running eluates. Compounds **3** and **4** display the following spectral data. **3:** ^{1}H NMR (DMSO-d_6) 1.49 (t, 3H, CH_3), 1.51 (t, 3H, CH_3), 4.48 (q, 2H, CH_2), 4.69 (q, 2H, CH_2), 7.75 (dd, 1H, ar, J = 8.8, 2.3 Hz), 8.02 (d, 1H, ar, J = 2.3 Hz), 8.36 (d, 1H, ar, J = 8.8 Hz); IR 1740. **4**: ^{1}H NMR (DMSO-d_6) 1.28 (t, 3H, CH_3), 1.39 (t, 3H, CH_3), 4.35−4.51 (m, 4H, $2CH_2$), 7.54 (dd, 1H, ar, J = 8.7, 1.9 Hz), 7.94 (d, 1H, ar, J = 1.9 Hz), 8.24 (d, 1H, ar, J = 8.7 Hz); IR 1690, 1735.

7-Chloro-5-*N*-ethyl-4,5-dihydro-4-oxo-1,2,4-triazolo[1,5-*a*]quinoxaline-2-carboxylic Acid (5). The title acid was obtained from **4** (0.46 mmol) as described for the synthesis of **2a**:[19] ^{1}H NMR (DMSO-d_6) 1.27 (t, 3H, CH_3), 4.38 (q, 2H, CH_2), 7.55 (dd, 1H, ar, J = 8.8, 1.8 Hz), 7.93 (d, 1H, ar, J = 1.8 Hz), 8.23 (d, 1H, ar, J = 8.8 Hz); IR 1730, 3100, 3420, 3540.

7-Chloro-4,5-dihydro-2-(hydroxymethyl)-1,2,4-triazolo-[1,5-*a*]quinoxalin-4-one (6). $LiBH_4$ (3.6 mmol) was carefully added to a stirred solution of **1b** (2.4 mmol) in anhydrous tetrahydrofuran (THF) (12 mL). The mixture was stirred at room temperature until the disappearance of the starting material (TLC monitoring) and by adding some more $LiBH_4$ (1.8 mmol each time) every 8 h. Evaporation of almost all the solvent gave a white suspension which was treated with HCl (2 N) until the end of the evolution of gas. After dilution with iced H_2O (5−6 mL) the solid was collected: ^{1}H NMR (DMSO-d_6) 4.69 (d, 2H, CH_2, J = 3.5 Hz), 5.62−5.69 (m, 1H, OH), 7.39−7.46 (m, 2H, ar), 8.06 (d, 1H, ar, J = 8.6 Hz), 12.43 (br s, 1H, NH); IR 1700, 3500.

2-(Bromomethyl)-7-chloro-4,5-dihydro-1,2,4-triazolo-[1,5-*a*]quinoxalin-4-one (7). PBr_3 (28 mmol) was added dropwise to a solution of **6** (2.8 mmol) in anhydrous THF (120 mL). The mixture was stirred at room temperature for 24 h and then diluted with iced H_2O (100 mL). The mixture was treated with a solution of NaOH (10%) until pH = 8−9 and extracted with ethyl acetate (100 mL × 3). The dried (Na_2SO_4) organic extracts were evaporated at reduced pressure to give a solid which was treated with Et_2O and collected: ^{1}H NMR (DMSO-d_6) 4.89 (s, 2H, CH_2), 7.40−7.46 (m, 2H, ar), 8.06 (d, 1H, ar, J = 8.4 Hz), 12.50 (s, 1H, NH); IR 1700.

7-Chloro-4,5-dihydro-4-oxo-1,2,4-triazolo[1,5-*a*]quinoxaline-2-acetonitrile (8). Compound **7** (1.5 mmol) was added portionwise to a solution of NaCN (1.7 mmol) in DMSO (10 mL). The yellow mixture was stirred at room temperature for 4 days and then diluted with iced H_2O (100 mL) and basified with a solution of NaOH (10%). The resulting solid was collected, washed with H_2O, and purified by column chroma-

tography, eluting system $CHCl_3$/MeOH (9:1). The central eluates were evaporated at reduced pressure to yield crude compound **8**. A second crop of **8** was obtained by extracting with ethyl acetate (50 mL × 3) the alkaline mother solution and evaporating at reduced pressure the organic solvent: ^{1}H NMR (DMSO-d_6) 4.52 (s, 2H, CH_2), 7.41−7.48 (m, 2H, ar), 8.01 (d, 1H, ar, J = 8.7 Hz), 12.50 (br s, 1H, NH); IR 1700, 2260.

Ethyl 7-Chloro-4,5-dihydro-4-oxo-1,2,4-triazolo[1,5-*a*]-quinoxaline-2-acetate (9). Concentrated H_2SO_4 (0.5 mL) was added dropwise to a solution of **8** (0.46 mmol) in EtOH (1 mL). The mixture was refluxed for 2.5 h, then cooled, and diluted with H_2O (10 mL). Neutralization of the solution with a saturated solution of $NaHCO_3$ yielded a solid which was collected and washed with H_2O: ^{1}H NMR (DMSO-d_6) 1.23 (t, 3H, CH_3), 4.06 (s, 2H, CH_2), 4.16 (q, 2H, CH_2), 7.41−7.46 (m, 2H, ar), 8.06 (d, 1H, ar, J = 8.5 Hz), 12.48 (br s, 1H, NH); IR 1700, 1735.

7-Chloro-4,5-dihydro-4-oxo-1,2,4-triazolo[1,5-*a*]quinoxaline-2-acetic Acid (10). A solution of NaOH (1 M, 0.5 mL) was added to a solution of **9** (0.16 mmol) in THF (5 mL). The mixture was stirred at room temperature for 24 h, then diluted with iced H_2O (15 mL), and acidified with glacial acetic acid. The mixture was extracted with ethyl acetate (25 mL × 3). The organic extracts were washed with H_2O (25 mL × 3), dried (Na_2SO_4), and evaporated at reduced pressure to produce a solid: ^{1}H NMR (DMSO-d_6) 3.95 (s, 2H, CH_2), 7.40−7.46 (m, 2H, ar), 8.06 (d, 1H, ar, J = 8.7 Hz), 12.47 (br s, 1H, NH), 12.73 (br s, 1H, COOH); IR 1605, 1700, 3400.

7-Chloro-4,5-dihydro-4-oxo-1,2,4-triazolo[1,5-*a*]quinoxaline-2-carbohydroxamic Acid (11). Hydroxylamine hydrochloride (10.2 mmol) was added to a solution of NaOH (10.2 mmol) in absolute EtOH (50 mL). The mixture was stirred at room temperature overnight. The solid was filtered off. Compound **1b** (1.02 mmol) and NaOH (10.2 mmol) were added to the solution which was then stirred at room temperature until disappearance of the staring material (TLC monitoring). Acidification with HCl (6 N) gave a solid which was filtered off. The solution was concentrated at reduced pressure to yield a solid which was collected: ^{1}H NMR (DMSO-d_6) 7.46 (dd, 1H, ar, J = 8.8, 2.2 Hz), 7.60 (d, 1H, ar, J = 2.2 Hz), 8.12 (d, 1H, ar, J = 8.8 Hz), 9.43 (br s, 1H, NH/OH), 11.84 (br s, 1H, OH/NH); IR 1720, 3100, 3200, 3440.

7-Chloro-4,5-dihydro-4-oxo-1,2,4-triazolo[1,5-*a*]quinoxaline-2-carbohydrazide (12). Hydrazine monohydrate (32 mmol) was added dropwise to a suspension of **1b** (1.7 mmol) in EtOH (20 mL). The resulting solid was collected and washed with H_2O: ^{1}H NMR (DMSO-d_6) 3.37 (br s, 2H, NH_2), 5.30 (br s, 1H, NH), 7.42−7.49 (m, 2H, ar), 8.13 (d, 1H, ar, J = 9.2 Hz), 12.50 (br s, 1H, NH); IR 1695, 1730, 3300.

7-Chloro-4,5-dihydro-4-oxo-1,2,4-triazolo[1,5-*a*]quinoxaline-2-carboxamide (13). A suspension of **2b** (2.6 mmol) in thionyl chloride (10 mL) was refluxed for 4 h. Evaporation at reduced pressure of the solvent yielded a residue which was washed twice with cyclohexane (30 mL) and immediately reacted with NH_4OH (30%, 50 mL) under stirring and cooling (ice bath). The mixture was then stirred at room temperature overnight. Dilution with H_2O (20 mL) yielded a solid which was collected and washed with H_2O: ^{1}H NMR (DMSO-d_6) 7.45−7.48 (m, 2H, ar), 7.92 (br s, 1H, NH), 8.13 (d, 1H, ar, J = 9.5 Hz), 8.33 (br s, 1H, NH), 12.50 (br s, 1H, NH); IR 1700, 3460.

General Procedure for the Preparation of Carboxamides 14−19. *N*-3-(Dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (2.26 mmol), 1-hydroxybenzotriazole (2.26 mmol), and the suitable amine (1.69 mmol) were successively added to a solution of **2b** (1.13 mmol) in anhydrous DMF (20 mL). The mixture was stirred at room temperature for 24 h. Compounds **14−17** and **19** were precipitated by diluting the solution with a small amount of H_2O, while compound **18** precipitated without dilution. Compounds **14−19** were collected and washed with H_2O. Compound **15** displays the following spectral data: ^{1}H NMR (DMSO-d_6) 4.51 (d, 2H, CH_2, J = 6.0 Hz), 7.25−7.49 (m, 7H, ar), 8.14 (d, 1H, ar, J = 9.1 Hz), 9.61 (t, 1H, NH, J = 6. 0 Hz), 12.52 (br s, 1H, NH); IR 1680, 1705, 3400.

***N*-(7-Chloro-4,5-dihydro-4-oxo-1,2,4-triazolo[1,5-*a*]quinoxalinyl-2-carbonyl)glycine Methyl Ester (20).** Triethylamine (1.08 mmol) was added to a solution of glycine methyl ester hydrochloride (1.08 mmol) in anhydrous DMF (20 mL). The mixture was stirred at room temperature for 10 min. Compound **2b** (0.9 mmol), *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (1.8 mmol), and 1-hydroxybenzotriazole (1.8 mmol) were successively added to the mixture, which was then stirred at room temperature for 15 h. Dilution with iced H_2O (80 mL) yielded a solid which was collected and washed with H_2O: ^{1}H NMR (DMSO-d_6) 3.69 (s, 3H, CH_3), 4.08 (d, 2H, CH_2, J = 5.6 Hz), 7.45−7.50 (m, 2H, ar), 8.14 (d, 1H, ar, J = 8.9 Hz), 9.37 (t, 1H, NH, J = 5.6 Hz), 12.50 (br s, 1H, NH); IR 1695, 1740, 3370.

***N*-(7-Chloro-4,5-dihydro-4-oxo-1,2,4-triazolo[1,5-*a*]quinoxalinyl-2-carbonyl)glycine (21).** A solution of NaOH (1 M, 1.8 mL) was added to a suspension of **20** (0.53 mmol) in THF/methanol (1:1, 10 mL). The mixture was stirred at room temperature overnight. The resulting solid was collected and dissolved in H_2O (10 mL). The solution was acidified with HCl (6 N) to afford a solid which was collected and washed with H_2O: ^{1}H NMR (DMSO-d_6) 3.97 (d, 2H, CH_2, J = 6.0 Hz), 7.45−7.50 (m, 2H, ar), 8.14 (d, 1H, ar, J = 8.4 Hz), 9.17 (t, 1H, NH, J = 6.0 Hz), 12.50 (br s, 1H, NH); IR 1690, 1740, 3240, 3360.

Biochemistry. Synaptic membrane preparation and [^{3}H]-glycine and [^{3}H]-DL-AMPA binding experiments were performed following described procedures.[26,27]

[^{3}H]-(+)-MK-801 Binding. [^{3}H]-(+)-MK-801 ((+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine maleate) binding assays were carried out according to Yoneda and Ogita[28] with slight modifications. Membranes were resuspended (0.5 mg of 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) and 10 μM glutamic acid in the presence and absence of the test compound in a total volume of 0.5 mL. Bound radioactivity was separated by filtration through GF/C filters presoaked in 0.05% poly(ethylenimine) 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 3−4 displacement curves based on 4−6 scalar concentrations of the test compound in triplicate using the ALLFIT computer program[29] and, in the case of tritiated glycine and AMPA binding, converted to K_i values by application of the Cheng−Prusoff equation.[30] In 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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