7-Chloro-4,5-dihydro-8-(1,2,4-triazol-4-yl)-4-oxo-1,2,4-triazolo[1,5-*a*]quinoxaline-2-carboxylates as Novel Highly Selective AMPA Receptor Antagonists

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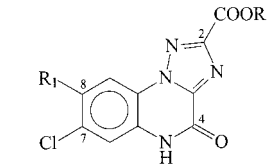
Introduction. Glutamate (Glu) is probably the major excitatory transmitter in the CNS, but it is also likely to be involved in many pathological processes. The excitotoxicity of Glu is mainly mediated by the overstimulation of the ionotropic Glu receptors (iGluRs): i.e., *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), and kainate (KA) receptors.[1,2] Accordingly, there has been a growing interest in the therapeutic potential of antagonists acting at these iGluRs.[3−6]

As part of a program aimed at finding novel iGluR antagonists, we have recently reported the synthesis of a set of 4,5-dihydro-4-oxo-1,2,4-triazolo[1,5-*a*]quinoxaline-2-carboxylates (TQXs).[7] The combined glycine/NMDA and AMPA receptor affinity of TQXs[7] suggested that this tricyclic system may represent a structure which, suitably modified, could lead to selective glycine/NMDA or AMPA receptor antagonists. We have hypothesized that combining the TQX framework with a 8-(imidazol-1-yl) substituent might produce selective AMPA antagonists. In fact, a pharmacophore model[6,8] of the AMPA receptor for the binding of quinoxalinedione and heterocyclic-fused quinoxalinone antagonists claimed that the imidazol-1-yl substituent was essential for high AMPA receptor activity. To verify this hypothesis, we have replaced the 8-nitro substituent of our mixed glycine/NMDA and AMPA antagonist 7-chloro-8-nitro-4-oxo-1,2,4-triazolo[1,5-*a*]quinoxaline-2-carboxylic acid (**1**)[7] with the bioisoster imidazol-1-yl moiety (compounds **2** and **3**, Chart 1). Moreover, although several nitrogen-containing heterocycles have been introduced as substituents on the benzo-fused moiety of AMPA antagonists,[9,10] to our knowledge the 1,2,4-triazol-4-yl substituent has never been considered in the structure−activity relationship (SAR) studies on quinoxalinone[9] and heterocyclic-fused quinoxalinone antagonists.[8] Thus, we synthesized and tested at iGluRs the 7-chloro-4,5-dihydro-8-(1,2,4-triazol-4-yl)-4-oxo-1,2,4-triazolo[1,5-*a*]quinoxaline-2-carboxylates **4** and **5** (Chart 1) to evaluate the influence of the 8-(1,2,4-triazol-4-yl) substituent at this crucial position in AMPA receptor recognition.

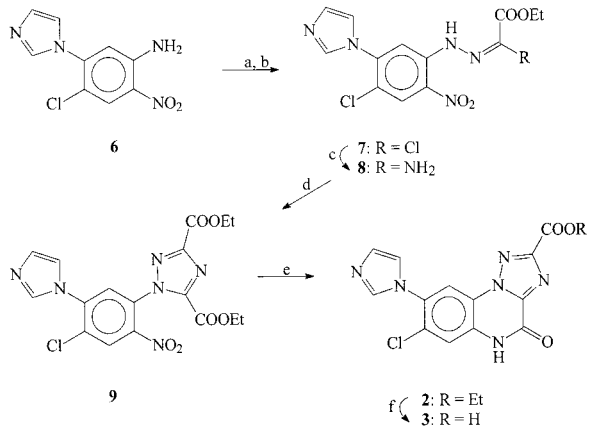
Chart 1



TQXs

	R	R_1
1	H	NO_2
2	Et	
3	H	
4	Et	
5 (TQX-173)	H	

Scheme 1[a]



[a] Reagents and conditions: (a) $NaNO_2/H_2SO_4$ concd, $NaBF_4$, 0−5 °C; (b) $CH_3COCHClCOOEt$, rt; (c) NH_3(g), anhydrous dioxane, rt, 1 h; (d) ClCOCOOEt, anhydrous toluene, reflux, 5 h; (e) iron, glacial acetic acid, 90 °C, 30 min; (f) 0.8 N NaOH, ethanol, rt, 1 h, then glacial acetic acid.

Results and Discussion. The 8-(imidazol-1-yl) derivatives **2** and **3** and the corresponding 8-(1,2,4-triazol-4-yl) analogues **4** and **5** were prepared following the two different synthetic strategies illustrated in Schemes 1 and 2. Briefly, by reacting the diazonium tetrafluoborate of 4-chloro-5-(imidazol-1-yl)-2-nitroaniline (**6**)[11] with ethyl 2-chloro-3-oxobutanoate the N^2-chloroacetate derivative **7** was prepared (59%) (Scheme 1). The latter was transformed with ammonia into its corresponding oxamidrazonate **8** (86%). Reaction of **8** with ethyloxalyl chloride directly yielded the diethyl 1-[4-chloro-5-(imidazol-1-yl)-2-nitrophenyl]-1,2,4-triazole-3,5-dicarboxylate (**9**) (51%). Reduction with iron and glacial acetic acid of the nitro group of **9** afforded the tricyclic ester **2** (85%) which was hydrolyzed to yield the 2-carboxylic acid **3** (92%).

In Scheme 2 the synthetic strategy for the preparation of the 8-(1,2,4-triazol-4-yl) derivatives **4** and **5** is illustrated. The starting material, ethyl 7-chloro-4,5-dihydro-4-oxo-1,2,4-triazolo[1,5-*a*]quinoxaline-2-carboxylate (**10**),[7] was regioselectively nitrated at position 8 with 90% HNO_3 to yield **11** (80%), which was reduced

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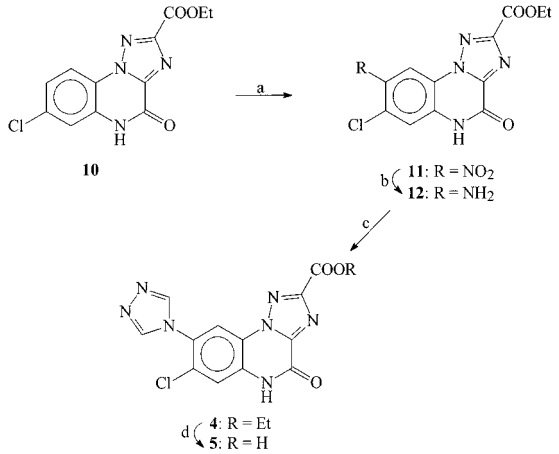
Scheme 2[a]

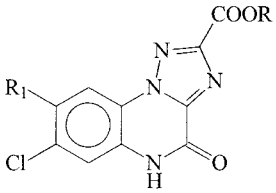


[a] Reagents and conditions: (a) 90% HNO_3, 0−5 °C, 5 h; (b) iron, glacial acetic acid, 90 °C, 1 h; (c) $(OHCNH)_2$, pyridine, Me_3SiCl, 100 °C, 15 h; (d) 0.8 N NaOH, ethanol, rt, 3 h, then 6 N HCl.

with iron and glacial acetic acid to its corresponding 8-amino derivative **12** (44%). By reacting **12** with diformylhydrazine, the ethyl 7-chloro-4,5-dihydro-8-(1,2,4-triazol-4-yl)-4-oxo-1,2,4-triazolo[1,5-*a*]quinoxaline-2-carboxylate (**4**) was isolated (73%). Alkaline hydrolysis of **4**, followed by acidification, yielded the target 2-carboxylic acid **5** (82%).

Compounds **2**−**5**, together with the previously reported **1**[7] and NBQX (2,3-dihydroxy-6-nitro-7-sulfamoylbenzo[*f*]quinoxaline) and DCKA (5,7-dichlorokynurenic acid) included as standard compounds, were tested for their ability to displace tritiated glycine,[12] AMPA,[13] and KA from their specific binding in rat cortical membranes. The binding results shown in Table 1 confirm our hypothesis that by combining the triazoloquinoxaline-2-carboxylate framework with the presence of a nitrogen-containing heterocycle at position 8 selective AMPA receptor antagonists could be obtained. In fact, while compound **1**[7] is a mixed glycine/NMDA and AMPA antagonist (see Table 1), the herein reported 8-heteroaryl derivatives **2**−**5** are AMPA-selective antagonists. In accordance with previously reported results[7] on the TQX series, the presence of a free carboxylic group at position 2 is not an important feature for anchoring to the AMPA receptor. In fact, the carboxylic acids **3** and **5** are only 2−5-fold more active at the AMPA receptor than their corresponding esters **2** and **4**, respectively. On the contrary, the binding data shown in Table 1 suggest that the presence of both a free 2-carboxylic group and a 8-heteroaryl substituent seems to be essential for KA receptor−ligand interaction. In fact, while the 8-nitro acid **1** and the 8-heteroaryl esters **2** and **4** are inactive as KA ligands, the 8-heteroaryl acids **3** and **5** display micromolar KA receptor affinities. It must be noted that the 8-(imidazol-1-yl) derivatives **2** and **3** are less active at the AMPA receptor than their corresponding 8-(1,2,4-triazol-4-yl) analogues **4** and **5**. These data show that the replacement of the imidazole moiety with a triazole one in the crucial position 8 of the TQX framework leads to an increase in AMPA receptor affinity. Moreover, the 8-(1,2,4-triazol-4-yl) derivatives **4** and **5** are not only more potent but also more selective AMPA antagonists than their corresponding 8-(imidazol-1-yl) derivatives **2** and **3**. In fact, the beneficial effect of the 8-(1,2,4-triazol-4-yl) moiety toward AMPA affinity and selectivity is indicated by the

Table 1. Binding Activity at Glycine/NMDA, AMPA, and KA Receptors and Functional Antagonism at NMDA and AMPA Sites[a]



compd	R	R_1	[^{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)[b] (or I%)[c]	Mouse cortical wedge preparation: IC_{50} (μM) *vs* agonist-induced depolarizations[d] AMPA	NMDA
1	H	NO_2	1.20 ± 0.08[e]	2.2 ± 0.3[e]	(41 ± 7%)	75 ± 14	75 ± 17
2	Et		2.30 ± 0.13	(39 ± 5%)	(24 ± 1%)	22 ± 3	>100
3	H		0.98 ± 0.04	(45 ± 5%)	16.8 ± 3.3	NT[f]	NT[f]
4	Et		0.70 ± 0.13	(15 ± 2%)	(42 ± 3%)	15 ± 2	100 ± 18
5 (TQX-173)	H		0.14 ± 0.02	33.5 ± 5.3	11.6 ± 1.3	2.3 ± 0.4	46 ± 4
NBQX			0.07 ± 0.06	(3.0 ± 0.2%)	7.0 ± 1.1	0.20 ± 0.02	(*)[g]
DCKA			(5.0 ± 0.5%)	0.09 ± 0.02	(8.0 ± 1%)	52 ± 11	4.7 ± 0.9

[a] 1 mM solutions of the tested compounds were prepared in DMSO/water (50%, v/v). [b] Inhibition constant (K_i) values and concentrations necessary for 50% inhibition of binding (IC_{50}) were means ± SEM of 3−4 separate determinations in triplicate. [c] Percentage of inhibition (I%) of specific binding at 100 μM concentration was based on 3 separate assays in triplicate. [d] Concentrations that inhibit by 50% depolarizations (IC_{50}) induced by 5 μM NMDA or AMPA were means ± SEM of 4 separate determinations. [e] Ref 7. [f] Due to the insolubility of the compound in the medium for electrophysiological assays, testing was not possible. [g] At 10 μM concentration the inhibition was not significant.

binding data of the triazole ester **4**, which is not only equi-active to the imidazole acid **3** but also more AMPA-selective than **3**.

Compounds **1**−**5** together with the well-known NBQX and DCKA were evaluated for functional antagonist activity by assessing their ability to inhibit depolarizations induced by 5 μM AMPA or NMDA in mouse cortical wedge preparations[14] (Table 1). Parallel experiments have demonstrated that the inhibitory actions of the tested compounds on AMPA- and NMDA-induced mouse cortical wedge depolarization are competitive with AMPA and glycine, respectively, since the actions are reversed by increasing concentrations of these agonists (data not shown).

In general, the results obtained in the electrophysiological assays closely correlate with the binding data on AMPA and glycine/NMDA receptors. In particular, the electrophysiological data confirm that the 7-chloro-4,5-dihydro-8-(1,2,4-triazol-4-yl)-4-oxo-1,2,4-triazolo[1,5-*a*]quinoxaline-2-carboxylic acid (**5**, TQX-173) is the most potent and selective AMPA antagonist herein reported. In fact, in agreement with [^{3}H]AMPA and [^{3}H]glycine binding results, the inhibitory action of **5** on depolarization induced by 5 μM AMPA ($IC_{50} = 2.3 \pm 0.4$ μM) was much higher than that on NMDA-evoked responses ($IC_{50} = 46 \pm 4$ μM).

In conclusion, the synthesis of these novel 8-heteroaryl TQXs **2**−**5**, their iGluR binding data, and the electrophysiological results have evidenced that the presence of the 8-(1,2,4-triazol-4-yl) moiety leads to more potent and selective AMPA antagonists than those bearing the claimed 8-(imidazol-1-yl) one.

Supporting Information Available: Experimental details, spectral data, and analytical data are available free of charge via the Internet at http://pubs.acs.org.

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