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3-Hydroxy-quinazoline-2,4-dione as a useful scaffold to obtain selective Gly/NMDA and AMPA receptor antagonists

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Abstract—The synthesis and Gly/NMDA, AMPA and KA receptor binding activities of some 3-hydroxy-quinazoline-2,4-dione derivatives are reported. The binding data, together with functional antagonism studies, showed that the 3-hydroxy-quinazoline-2,4-dione moiety can be considered a useful scaffold to obtain selective Gly/NMDA and AMPA receptor antagonists. In fact, introduction of chlorine atom(s) on precise position(s) of the benzofused moiety yielded Gly/NMDA selective antagonists, while the presence of the 6-(1,2,4-triazol-4-yl) group shifted the affinity and selectivity towards the AMPA receptor.

Glutamate (Glu), the major excitatory neurotransmitter in the mammalian central nervous system, exerts its physiological roles via metabotropic (mGluRs) and ionotropic receptors (iGluRs). The iGluRs, ligand-gated ion channels, are subdivided into three major subclasses, namely *N*-methyl-D-aspartate (NMDA), (*S*)-2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)propionic acid (AMPA) and kainate (KA) receptors.[1,2] The NMDA receptor complex possesses different binding sites including the glycine co-transmitter receptor (Gly/NMDA).[3] It is well known that overstimulation of iGluRs is involved in acute and chronic degenerative disorders such as stroke, ischaemia, Parkinson's and Alzheimer's diseases, epilepsy and pain.[4–6] Accordingly, iGluR antagonists, as well as the Gly/NMDA receptor antagonists are under investigation for their potential therapeutic use in the above mentioned pathologies.

In recent years, in our laboratory much effort has been directed towards the study of novel competitive and uncompetitive iGluR antagonists in order to shed light on the different structural requirements of the diverse receptor types.[7–13] In particular, we prepared a series of 3-aryl-quinazoline-2,4-dione derivatives[7] (Chart 1) some of which showed a binding affinity at the Gly/NMDA receptors in the micromolar range. In fact, these derivatives possess all the pharmacophoric descriptors of the Gly/NMDA receptor antagonists proposed by Leeson and Iversen:[14] (a) a flat hydrophobic area, profitably substituted with chlorine atoms, represented by the fused benzo ring; (b) a NH hydrogen-bond donor that binds a proton acceptor of the receptor; (c) a δ-negatively charged carbonyl oxygen atom that forms a coulombic interaction with a positive receptor site; (d) a hydrogen-bond acceptor, represented by the 4-carbonyl group, able to interact with a receptor hydrogen-bond donor. Moreover, an attractive interaction between the 3-aryl ring and the positively charged receptor site could be hypothesized.

Thus, to continue the study of this class of derivatives and to ameliorate their affinity, we replaced the 3-aryl ring with a 3-hydroxy substituent (compounds **1**–**6**, Chart 1). In fact, it seemed obvious that the acidic 3-hydroxy group could engage, with the positively charged receptor site, a stronger interaction, compared to that formed by the 3-aryl ring. Moreover, the less hindered 3-hydroxy group could make the approach to the positively charged receptor subsite easier. Finally, this substitution could improve the water solubility of these compounds.

Keywords: Ionotropic glutamate receptors; Glycine/NMDA receptor antagonists; AMPA receptor antagonists; Quinazoline-2,4-dione derivatives.
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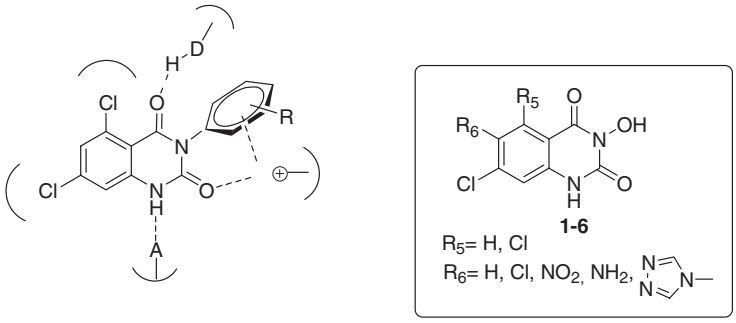
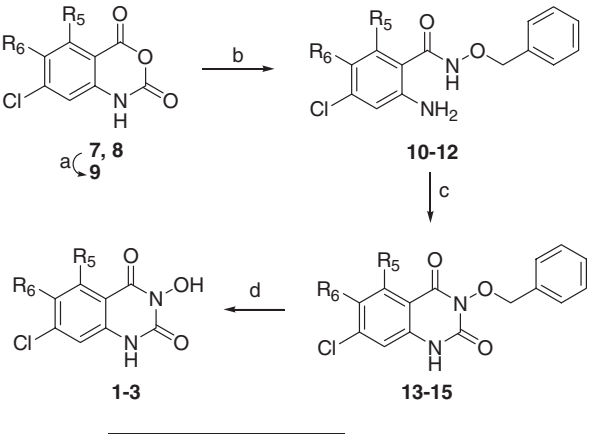


Chart 1. Previously and currently reported quinazoline-2,4-dione derivatives.

Therefore, compounds **1**–**6** were prepared and tested at the Gly/NMDA site. In particular, in this preliminary work, we introduced chlorine atom(s) on precise position(s) of the fused benzene ring (compounds **1**–**3**) since it is well known that their presence increases the affinity of several classes of Gly/NMDA receptor antagonists. Compounds **1**–**6** were also tested at the AMPA receptor. In fact, it has to be noted that **1**–**6** also possess the structural requirements described by Bigge and Nikam in the AMPA receptor pharmacophore model proposed for the quinoxaline-2,3-dione derivatives.[4] Therefore, we rationally designed the 7-chloro-6-(1,2,4-triazol-4-yl) derivative **6**, since, from our experience with 1,2,4-triazolo[1,5-*a*]quinoxalin-4-one derivatives,[9,11,13] the presence of this heterocycle at such a position could be particularly profitable for AMPA receptor affinity and selectivity. Finally, we also evaluated the binding affinity of derivatives **1**–**6** at KA receptor to obtain information about the structural requirements of this still little known iGluR subtype.

Compounds **1**–**6**[15] were prepared as depicted in Schemes 1 and 2. The 1,2-dihydro-3,1-benzoxazine-2,4-diones **7** and **8**, bearing the 7-chloro and 5,7-dichloro substituents, respectively, were prepared as previously described.[7,16] The 6,7-dichloro-derivative **9** was prepared by chlorination of **7** with SO_2Cl_2 in glacial acetic acid and catalytic amount of iodine, differently from the previously reported method.[17] The 2-amino-*N*-benzyloxybenzamides **10**–**12** were prepared by allowing **7**–**9** to react with *O*-benzylhydroxylamine in refluxing ethanol. Cyclization of **10**–**12** with triphosgene afforded the 3-benzyloxy-quinazoline-2,4-dione derivatives **13**–**15**, which were debenzylated with 48% HBr in glacial acetic acid to give the desired 3-hydroxy-quinazoline-2,4-diones **1**–**3**. The 3-hydroxy derivative **1** was transformed into the corresponding 3-acetate **16** by refluxing in acetic anhydride (Scheme 2). Derivative **16** was regioselectively nitrated to afford the 6-nitro compound **17**. Desacetylation of **17** with an aqueous NaOH solution followed by acidification yielded the 7-chloro-3-hydroxy-6-nitro-quinazoline-2,4-dione **4**. Catalytical reduction of **17** gave the 6-amino derivative **18**. This compound was hydrolyzed to the final 6-amino-7-chloro-3-hydroxy-quinazoline-2,4-dione **5**. Reaction of derivative **18** with diformylhydrazine afforded compound **19**, which was desacetylated to give the desired 7-chloro-3-hydroxy-6-(1,2,4-triazol-4-yl)-quinazoline-2,4-dione **6**.



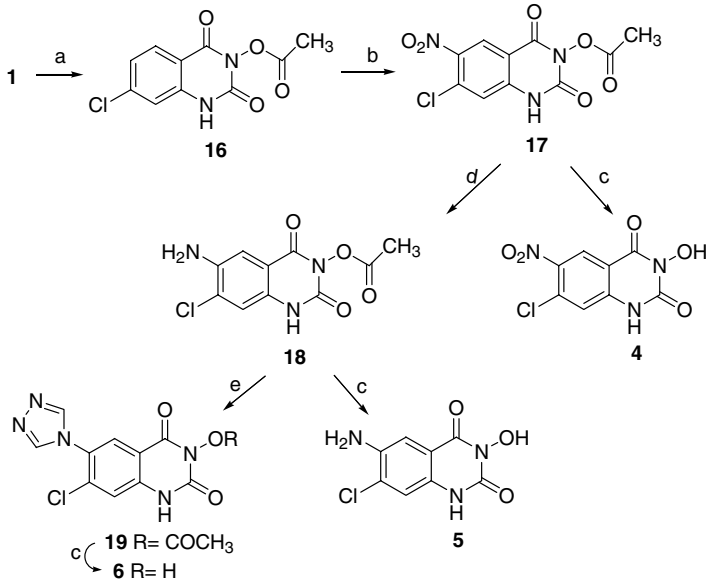
	R_5	R_6
1, 7, 10, 13	H	H
2, 8, 11, 14	Cl	H
3, 9, 12, 15	H	Cl

Scheme 1. Reagents and conditions: (a) SO_2Cl_2, glacial AcOH, I_2, 50 °C; (b) $PhCH_2ONH_2 \cdot HCl$, NEt_3, EtOH, reflux; (c) Triphosgene, THF, NEt_3, rt; (d) 48% HBr, AcOH, reflux.

The binding affinities of compounds **1**–**6** at the Gly/NMDA and AMPA receptors were evaluated by measuring their ability to displace [^{3}H]glycine and [^{3}H]AMPA, respectively, from rat cerebral cortex synaptic membranes, following the procedure described in Ref. 7 and 18. The high affinity [^{3}H] kainate binding assays were performed on rat cortical membrane, according to a previously described method.[12]

The Gly/NMDA binding data of **1**–**6**, reported in Table 1 together with that of the previously described 3-phenyl-quinazolin-2,4-dione **A**[7], confirm our working hypothesis. Replacement of the 3-aryl moiety of the previously reported quinazoline-2,4-dione derivatives with the 3-hydroxy group resulted in a significant enhancement of the Gly/NMDA receptor affinity (compare **2** with **A**). Indeed, compounds **1**–**3**, designed as Gly/NMDA ligands, show an affinity towards this receptor in the low micromolar range. Moreover, **1**–**3** also possess a good selectivity for the Gly/NMDA site towards both AMPA receptor (from 40- to 50-fold) and KA receptor (more than 220-fold).

Derivative **6**, designed as an AMPA selective antagonist, is very interesting. It shows not only a good AMPA

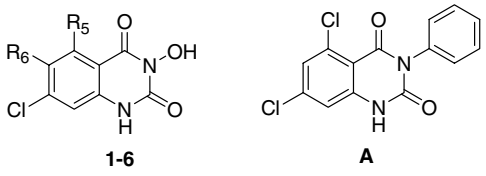


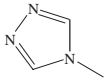
Scheme 2. Reagents and conditions: (a) Refluxing Ac_2O; (b) 90% HNO_3, −10 °C; (c) (i) 2.5% NaOH, 40 °C; (ii) 6 N HCl; (d) 10% Pd/C, EtOAc, 40 psi; (e) Diformylhydrazine, Me_3SiCl, pyridine, NEt_3, 100 °C.

receptor affinity, but also a high selectivity, displaying more than 400-fold higher affinity for the AMPA than for the Gly/NMDA receptor. These data, firstly, confirm the advantageous effect of the 6-(1,2,4-triazol-4-yl) moiety for AMPA receptor affinity and selectivity, which as previously hypothesized,[9,11] could be due to a hydrogen bond that the N^3 atom of the triazole ring form with a putative hydrogen-bond donor of the receptor. Secondly, the binding data of **6** indicate that the fused benzo ring of these 3-hydroxy-quinazoline-2,4-dione derivatives interacts with a large lipophilic pocket of the AMPA receptor, which well tolerates a bulky group, such as the 6-(1,2,4-triazol-4-yl) ring. On the contrary, the corresponding lipophilic area of the Gly/NMDA receptor is a size-limited binding pocket which does not permit the anchoring of a bulky 6-substituent. All these findings agree well with our previous results obtained for the 1,2,4-triazolo[1,5-*a*]quinoxalin-4-one derivatives[8,9,11–13] and confirm the structural requirements proposed in the AMPA and Gly/NMDA receptor antagonist pharmacophore models.[4,14]

Introduction of a nitro group at the 6-position of the 7-chloro derivative **1** reduces the Gly/NMDA affinity

Table 1. Binding activity at Gly/NMDA, AMPA and KA receptors



Compound	R_5	R_6	K_i (μM)[a]		IC_{50} (μM)[b]
			[^{3}H]glycine	[^{3}H]AMPA	[^{3}H]KA
1	H	H	0.24 ± 0.02	11.6 ± 1.9	140 ± 15
2	Cl	H	0.20 ± 0.03	9.8 ± 1.4	52 ± 4
3	H	Cl	0.3 ± 0.03	12.5 ± 2.6	68 ± 7
4	H	NO_2	1.1 ± 0.1	1.3 ± 0.1	19.2 ± 1
5	H	NH_2	10.6 ± 1.2	9.7 ± 1.5	70 ± 6
6	H		>100	0.25 ± 0.04	7.0 ± 0.5
A[c]			23 ± 6	NT[d]	NT[d]

[a] K_i values were means ± SEM of three or four separate determinations in triplicate.
[b] IC_{50} values were means ± SEM of three or four separate determinations in triplicate.
[c] Ref. 6.
[d] Not tested.

while it increases that towards the AMPA receptor, thus leading to a nonselective Gly/NMDA versus AMPA antagonist (compound **4**). The lower Gly/NMDA affinity of **4**, with respect to **1**, confirms the negative effect of a 6-substituent bulkier than a chlorine atom for anchoring to the Gly/NMDA receptor. The 10-fold increased AMPA affinity of **4**, compared to that of **1**, is consistent with the hypothesis of a hydrogen bonding interaction at the level of the 6-substituent. Nevertheless, the profitable effect of the 6-nitro group probably also arises from its electron-withdrawing properties that increases the NH acidity, thus reinforcing the hydrogen-bonding interaction with the receptor proton donor site.[4,11] Reduction of the 6-nitro group of **4** gave the 6-amino derivative **5**, which compared to the former, displays a reduced affinity for both the Gly/NMDA and AMPA receptor. As regards the binding data of **1**–**6**, at the high affinity KA receptor, resemble those at the AMPA receptor, even if their values are from 5- to 30-fold lower. In particular, compound **6**, the most active at the AMPA receptor, also shows the highest KA affinity, among the herein reported ligands.

In order to assess the functional antagonism at the NMDA receptor–ion channel complex, we tested derivatives **1**–**6** for their ability to inhibit the binding of the channel blocking agent [^{3}H]-(+)-MK-801((+)-5-methyl-10,11-dihydro-5*H*-benzo[*a*,*d*]cyclohepten-5,10-imine)[10,19,20] in rat cortical membranes incubated with 10 μM glutamate and 0.1 μM glycine. The results obtained are listed in Table 2. In general, the IC_{50} values of these derivatives closely correlate with their K_i values on [^{3}H]glycine binding, the only exception being compound **1**, which, in this assay, results about 6-fold less active than derivatives **2** and **3**, which both show binding activity similar to **1**.

The selected 3-hydroxy-quinazoline-2,4-dione derivatives **2**, **4**, **6**, together with NBQX (2,3-dihydroxy-6-nitro-7-sulphamoylbenzo[*f*]quinoxaline) and DCKA (5,7-dichlorokynurenic acid), as reference compounds, were evaluated for functional antagonist activity by assessing their ability to inhibit depolarization induced by 5 μM AMPA or NMDA in mouse cortical wedge preparations[21] (Table 3). The electrophysiological assays were performed following the procedures described in Ref. 12. All the tested compounds inhibited AMPA and NMDA responses in a reversible manner. The electrophysiological potencies of compounds **4** and **6** closely correlate with their binding affinities at the Gly/NMDA and AMPA receptors. In fact, **4** shows similar potencies in the Gly/NMDA and AMPA functional assays while the inhibitory action of **6** on depolarization induced by AMPA was higher than that on NMDA-evoked response. On the contrary, the functional antagonist activity of compound **2** versus NMDA receptor is not in agreement with the [^{3}H]glycine binding result since **2** shows about 50-fold higher affinity on the Gly/NMDA site than on AMPA receptor, while, it appears equipotent in inhibiting NMDA- and AMPA-evoked responses.

Table 2. Inhibition of stimulated [^{3}H]-(+)-MK-801 binding

Compound	IC_{50} (μM)[a] or I%[b]
1	4.8 ± 0.7
2	0.75 ± 0.08
3	0.43 ± 0.1
4	1.7 ± 0.01
5	89 ± 9
6	29%

[a] Concentration giving 50% inhibition of stimulated [^{3}H]-(+)-MK-801 binding. All assays were carried out in the presence of 10 μM glutamate and 0.1 μM glycine. IC_{50} values were means ± SEM of three or four separate determinations in triplicate.

[b] Percentage of inhibition (I%) of specific binding at 100 μM concentration.

Table 3. Functional antagonism at NMDA and AMPA sites of some selected quinazoline-2,4-diones

Compound	IC_{50} (μM)[a]	
	NMDA	AMPA
2	42 ± 5	33 ± 4
4	36 ± 4	29 ± 2
6	84 ± 10	4.6 ± 0.5
DCKA	4.7 ± 0.9	52 ± 11
NBQX	(*)[b]	0.20 ± 0.02

[a] Concentration necessary for 50% inhibition (IC_{50}) of depolarization induced by 5 μM S-AMPA or NMDA in mouse cortical wedge preparation. IC_{50} values were means ± SEM of three separate determinations.

[b] At 10 μM concentration the inhibition was not significant.

In conclusion, the herein reported preliminary study has identified the 3-hydroxy-quinazoline-2,4-dione as a new scaffold to obtain selective Gly/NMDA and AMPA receptor antagonists. In fact, introduction of suitable substituents on the benzofused moiety have led to either Gly/NMDA or AMPA selective antagonists. Moreover, this work has confirmed some different structural requirements of the Gly/NMDA and AMPA receptors. Taking into account these findings, further modifications of these derivatives, to improve both affinity and selectivity, are in progress.

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