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Original article

3-Hydroxy-1*H*-quinazoline-2,4-dione derivatives as new antagonists at ionotropic glutamate receptors: Molecular modeling and pharmacological studies

Vittoria Colotta [a,*], Ombretta Lenzi [a], Daniela Catarzi [a], Flavia Varano [a], Lucia Squarcialupi [a], Chiara Costagli [b], Alessandro Galli [b], Carla Ghelardini [b], Anna Maria Pugliese [b], Giovanna Maraula [b], Elisabetta Coppi [b], Domenico E. Pellegrini-Giampietro [b], Felicita Pedata [b], Davide Sabbadin [c], Stefano Moro [c]

[a] *Dipartimento di Scienze Farmaceutiche, Laboratorio di Progettazione, Sintesi e Studio di Eterocicli Biologicamente Attivi, Universita' di Firenze, Polo Scientifico, Via Ugo Schiff 6, 50019 Sesto Fiorentino, FI, Italy*

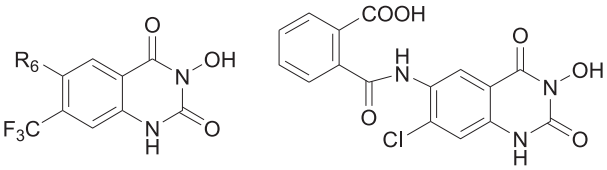
[b] *Dipartimento di Farmacologia Preclinica e Clinica, Università degli Studi di Firenze, Viale Pieraccini 6, 50139 Firenze, Italy*

[c] *Molecular Modeling Section (MMS), Dipartimento di Scienze del Farmaco, Università degli Studi di Padova, via Marzolo 5, 35131 Padova, Italy*

HIGHLIGHTS

- Heterocyclic rings at the 6-position gave good AMPA receptor affinity.
- Derivative **8** (R_6 = 1,2,4-triazol-4-yl) showed high AMPA receptor affinity.
- Derivative **3** showed the best affinity and selectivity for the kainate receptor.
- Derivatives **3** and **8** were effective neuroprotective agents in rat models of cerebral ischemia.
- Compound **8** showed anticonvulsant effect in pentylenetetrazole-induced convulsions.

GRAPHICAL ABSTRACT



R_6= H, NO_2, NH_2, Heterocycle, 2-COOH-C_6H_4-CONH

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ABSTRACT

Based on our 3-hydroxy-7-chloroquinazoline-2,4-dione derivatives, previously reported as antagonists at ionotropic glutamate receptors, we synthesized new 3-hydroxyquinazoline-2,4-diones bearing a trifluoromethyl group at the 7-position and different groups at position 6. Glycine/NMDA, AMPA and kainate receptor binding data showed that the 7-trifluoromethyl residue increased AMPA and kainate receptor affinity and selectivity, with respect to the 7-chlorine atom. Among the probed 6-substituents, the 6-(1,2,4-triazol-4-yl) group (compound **8**) was the most advantageous for AMPA receptor affinity and selectivity. Derivative **8** demonstrated to be effective in decreasing neuronal damage produced by oxygen and glucose deprivation in organotypic rat hippocampal slices and also showed anticonvulsant effects in pentylenetetrazole-induced convulsions. The previously reported kainate receptor antagonist 6-(2-carboxybenzoyl)-amino-7-chloro-3-hydroxyquinazoline-2,4-dione **3** prevented the failure of neurotransmission induced by oxygen and glucose deprivation in the CA1 region of rat hippocampal slices.

Abbreviations: Glu, glutamate; iGluRs, ionotropic glutamate receptors; NMDA, *N*-methyl-D-aspartate; AMPA, (*S*)-2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)propionic acid; KA, kainate; DCKA, 5,7-dichlorokynurenic acid; NBQX, 2,3-dihydroxy-6-nitro-7-sulphamoyl-benzo[*f*]quinoxaline; MK-801, (+)-5-methyl-10,11-dihydro-5*H*-benzo[*a*,*d*]cyclohepten-5,10-iminemaleate; PTZ, pentylenetetrazole; OGD, oxygen and glucose deprivation; PI, propidium iodide; fepsp, field excitatory postsynaptic potential; AD, anoxic depolarization; aCSF, artificial cerebrospinal fluid.

* Corresponding author. Tel.: +39 055 4573731; fax: +39 055 4573780.
E-mail address: vittoria.colotta@unifi.it (V. Colotta).

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1. Introduction

Glutamate (Glu) is the major excitatory neurotransmitter in the mammalian central nervous system and plays key roles in regulating many physiological processes through activation of metabotropic (mGluRs) and ionotropic receptors (iGluRs) [1,2]. The iGluRs include three families classified as *N*-methyl-D-aspartate (NMDA), (*S*)-2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl) propionic acid (AMPA) and kainate (KA) receptors. The NMDA receptor complex possesses different binding sites including the glutamate co-agonist glycine binding site (Gly/NMDA) [2]. To date, seven NMDA receptor subunits (GluN1, GluN2A-2D, and GluN3A and GluN3B), four AMPA receptor subunits (GluA1-GluA4) and five KA receptor subunits (GluK1-GluK5) have been cloned and characterized [2]. It is well known that an overstimulation of iGluRs induce an increase of intracellular Ca^{2+} concentrations, release of K^+ into the extracellular space and cell swelling due to the passive movement of water with Na^+ influx [3]. The massive increase of intracellular Ca^{2+} triggers numerous deleterious processes, including free radical formation and membrane degradation, mitochondrial dysfunction, inflammation, DNA-damage and apoptosis. These processes lead to neuronal death and are implicated in many diseases [1] such as Alzheimer's [4] and Parkinson's diseases [5], amyotrophic lateral sclerosis [6], epilepsy [1,7] and cerebral ischemia [8].

Due to the iGluR-mediated excitotoxicity, several compounds acting as iGluR antagonists demonstrated beneficial effects against the above cited pathologies [1,4,5].

It has to be noted that AMPA and KA receptor antagonists seem to possess greater therapeutic potential than NMDA antagonists because these latter often show adverse effects such as hallucinations, agitation, ataxia and catatonia [9].

In the last decade, a part of our research has been focused on the study of competitive and noncompetitive iGluR antagonists, belonging to different heteroaromatic systems [10–21]. Among them, the 3-hydroxyquinazoline-2,4-dione was disclosed as a useful scaffold to obtain selective iGluR antagonists [15,18], since many derivatives of this series showed affinity for AMPA and KA receptors or for the Gly/NMDA site. The 3-hydroxyquinazolin-2,4-diones possess the most important structural requirements for Gly/NMDA and AMPA or KA receptor recognition [22,23]: a flat hydrophobic area represented by the fused benzo ring; a NH hydrogen-bond donor that binds a proton acceptor of the receptor; a δ-negatively charged moiety, represented by both the 2-carbonyl group and the 3-hydroxy substituent, able to form a hydrogen bond with a cationic hydrogen bond donor receptor site. All the previously described 3-hydroxyquinazoline-2,4-dione derivatives (Fig. 1) are substituted at the 7-position with a chlorine atom that is combined with different substituents at the 6-position. Structure–affinity relationship (SAR) studies suggested that the nature of the 6-substituents was critical for the selectivity towards the different iGluRs. In particular, $R_6 = H$ gave a selective Gly/NMDA antagonist (compound **1**), while heterocyclic rings gave AMPA receptor antagonists, the most advantageous was the 6-(1,2,4-triazol-4-yl) moiety (derivative **2**) [15]. Instead, the presence of a 6-(2-carboxybenzoyl)-amino group on the 3-hydroxyquinazoline-2,4-dione scaffold afforded a selective KA receptor antagonist (compound **3**) [18]. On the basis of these results, we decided to continue the study of this class of compounds to further investigate the SARs and to obtain more potent AMPA and/or KA receptor antagonists. Thus, we planned the synthesis of new 6-substituted 3-hydroxyquinazoline-2,4-dione derivatives (Fig. 2, compounds **4**–**11**) in which the 7-chlorine atom was replaced with a 7-trifluoromethyl residue that was thought to increase affinity towards AMPA and KA receptors [12,16]. The 6-position of the new derivatives bears the substituents

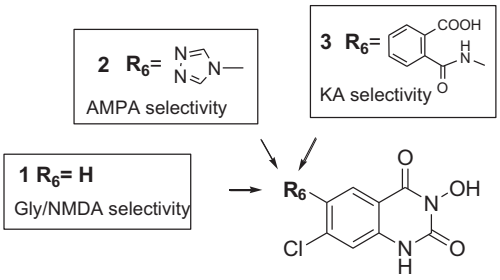


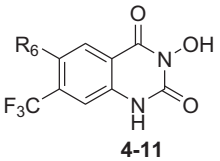
Fig. 1. Previously reported 7-chloro-3-hydroxyquinazoline-2,4-dione derivatives.

which in previous studies turned out to be profitable for the AMPA or KA receptor affinity and selectivity, i.e. heterocyclic rings or the 2-carboxybenzoylammino group, respectively.

2. Chemistry

The target compounds **4**–**11** were synthesized as described in Schemes 1 and 2. The 7-trifluoromethyl-1,2-dihydro-3,1-benzoxazine-2,4-dione **12** [21] was reacted with O-benzylhydroxylamine in refluxing ethanol to give the 2-amino-*N*-benzyloxy-4-trifluoromethylbenzamide **13**. Cyclization of **13** with triphosgene afforded the 3-benzyloxy-7-trifluoromethylquinazoline-2,4-dione **14** which was debenzylated with 48% HBr in glacial acetic acid to give the desired 3-hydroxy derivative **4**. This latter was transformed into the corresponding 3-acetate **15** by refluxing in acetic anhydride. Nitration of **15** with 90% HNO_3 afforded the corresponding 6-nitro compound **16** that was transformed into the 6-amino derivative **17**. Reaction of **17** with phthalic anhydride afforded the desired 3-hydroxy-6-(2-carboxybenzoylamino)-quinazoline-2,4-dione **7**. The 3-hydroxy-6-nitroquinazoline-2,4-dione **5**, its corresponding 6-amino derivative **6** and the 6-heteroaryl substituted compounds **8**–**11** were synthesized as shown in Scheme 2. Derivative **12** was reacted with O-methylhydroxylamine to yield the 2-amino-3-methoxy-4-trifluoromethylbenzamide **18** which was cyclized with triphosgene to give derivative **19**. Nitration of **19** afforded 6-nitro-3-methoxy derivative **20** which was desmethylated with 48% hydrobromic acid in glacial acetic acid to give the 3-hydroxy-6-nitroquinazoline-2,4-dione **5**. Catalytic reduction (Pd/C) of compound **5** gave the 6-amino derivative **6**.

To prepare the 7-trifluoromethyl-3-hydroxy-6-(1,2,4-triazol-4-yl)-quinazoline-2,4-dione **8**, compound **20** was reduced to the corresponding 6-amino compound **21** that was transformed into **22** by reaction with diformylhydrazine. Different attempts to turn the 3-O-methyl derivative **22** into the corresponding 3-hydroxy derivative **8** failed. In fact, treatment of **22** with boron tribromide at room temperature for three to four days left compound **22** unmodified, while reaction with 48% aqueous hydrobromic acid in glacial acetic acid at 100 °C afforded the 6-amino compound **6**. Thus, the desired derivative **8** was obtained by reacting compound **6** with



4-11

R_6= H, NO_2, NH_2, Heterocycle, 2-COOH-C_6H_4-CONH

Fig. 2. Currently reported 3-hydroxyquinazoline-2,4-dione derivatives.