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Original article

Synthesis and pharmacological studies at the Gly/NMDA, AMPA and Kainate receptors of new oxazolo[4,5-c]quinolin-4-one derivatives bearing different substituents at position-2 and on the fused benzo ring

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Abstract

The synthesis and biological evaluation at the Gly/NMDA, AMPA and Kainate receptors of new oxazolo[4,5-c]quinolin-4-one derivatives are reported. Different substituents were introduced at the 2-position (mercapto, carbonyl and methyl groups) and on the fused benzo ring (chlorine atom(s) and trifluoromethyl group). Among the herein reported compounds, the 2-mercapto-derivatives **1–4** showed the highest Gly/NMDA affinities, comparable to that of 5,7-dichlorokynurenic acid. The most active compound was the 7-chloro-substituted derivative **1** (K_i = 0.082 μM) which possesses a Gly/NMDA selectivity of 50- and 500-fold with respect to AMPA and KA receptors, respectively. Functional antagonism studies performed on some selected 2-mercapto compounds, at both AMPA and NMDA receptor-ion channels, assessed the antagonistic properties of these derivatives. SAR studies pointed out the importance of the concurrent presence of electron-rich moieties at both the 2- and 3-positions of the oxazolo[4,5-c]quinolin-4-one framework. In fact, the 3-sp^2-nitrogen atom plays a significant role in reinforcing the hydrogen bond that the 4-carbonyl oxygen probably forms with the arginine residue (R523) of the Gly/NMDA receptor site. The presence of 2-substituent able to form a hydrogen bonding interaction was also proved to be important for a good Gly/NMDA receptor affinity.

Keywords: Oxazoloquinoline derivatives; Ionotropic glutamate receptor antagonists; Glycine/NMDA receptor antagonists; AMPA receptor antagonists

1. Introduction

The excitatory neurotransmitter glutamate (Glu) plays an essential role in many physiological functions of the mammalian central nervous system where it activates two receptor classes: the metabotropic and ionotropic receptors. The ionotropic receptors (iGluRs) are ligand-gated ion channels, permeable to Na^+, K^+ and Ca^{2+} ions, and are presently classified into three major subclasses: *N*-methyl-D-aspartate (NMDA) (S)-2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)-propionic acid (AMPA) and kainate (KA) receptors [1,2]. On the NMDA receptor complex different binding sites are present including the glycine (Gly/NMDA) receptor. Binding of glycine to this site is necessary for activation of the NMDA receptor itself [3]. In the last few years it has been well established that overstimulation of iGluRs and the subsequent excess of intracellular Ca^{2+} are involved in many pathological conditions such as Parkinson's, Alzheimer's and Huntington's diseases, stroke, cerebral ischemia and pain [1,4–8]. As a consequence, iGluRs antagonists have been considered attractive targets for their potential therapeutic use in the above cited pathologies. While competitive NMDA receptor antagonists have shown many side effects, such as ataxia, neurotoxicity, memory deficits, psichotomimetic effects, the Gly/NMDA receptor antagonists have demonstrated to possess fewer adverse reactions [9–11]. Thus, in the last few years much effort has been directed towards the study of Gly/NMDA receptor antagonists belonging to various chemical classes. Among them, different series of tricyclic derivatives have been developed [1,9–12]. As a part of a program aimed at finding novel iGluR receptor antagonists [13–17],

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we have published some papers on 4-oxo-1,2,4-triazolo[1,5-a]quinoxaline-2-carboxylate derivatives (TQX series) [13] and 5-oxo-pyrazolo[1,5-c]quinazoline-2-carboxylate compounds (PQZ series) [17] (Fig. 1) some of which showed significant Gly/NMDA affinity. Indeed, derivatives of both series possess some important structural requirements of Gly/NMDA receptor antagonists [11,18]: (a) a flat hydrophobic area represented by the fused benzo ring; (b) a NH hydrogen bond donor that binds a proton acceptor of the receptor; and (c) a δ-negatively charged moiety, represented by both the carbonyl group, adjacent to the NH proton donor, and the 3-nitrogen atom, which could form a hydrogen bond with a cationic hydrogen bond donor–receptor site. Structure–activity relationship (SAR) studies in the TQX and PQZ series have pointed out the importance of the ethyl 2-carboxylate function or, even better, of the acidic 2-carboxyl group for anchoring to the Gly/NMDA binding site. The positive effect of both these substituents has been attributed to their capability of interacting with a putative hydrogen bond donor–receptor site [13,17]. Electron-withdrawing substituents, such as chlorine atom(s) and a trifluoromethyl group, on precise position(s) of the fused benzo ring have also been profitable for Gly/NMDA affinity of TQX and PQZ derivatives. On this basis and with the purpose of extending our investigation to other tricyclic iGluR antagonists strictly correlated to the TQX and PQZ series, we designed the synthesis of new compounds, namely the oxazolo[4,5-c]quinolin-4-one derivatives **1–12** (Fig. 2). The 2-mercapto-compounds **1–4** still maintain the important pharmacophoric descriptors described above: (i) the NH proton donor, (ii) the δ-negatively charged N-3 and 4-carbonyl oxygen atom, (iii) the 2-substituent which can engage a hydrogen bond, acting as a proton acceptor or, more likely, as proton donor, and (iv) the chlorine atom(s) or trifluoromethyl substituent on suitable position(s) of the fused benzo ring. The choice of introducing the 2-mercapto group in compounds **1–4** was made since to our knowledge this substituent has never been investigated in analogous tricyclic Gly/NMDA receptor antagonists. Subsequently, the 2-mercapto group was replaced by the 2-oxo function (derivatives **5–8**): this latter group is able to form a hydrogen bond acting as a proton acceptor group. Moreover, introduction of the 2-C=O group implied replacement of the 3-nitrogen atom with a NH moiety which was likely to interact with different strength with the cationic hydrogen bond donor–receptor site. However, since some 2,3,4,5-tetrahydro-oxazolo[4,5-c]quinoline-2,4-diones, bearing an 8-heteroaryl substituent, were already described as AMPA receptor antagonists [19], it was predictable that compounds **5–8** would possess Gly/NMDA affinity, being similar the structural requirements of these two receptors [18].

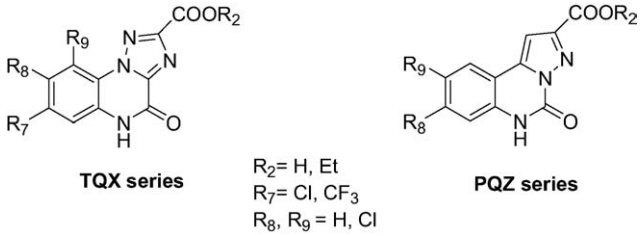


Fig. 1. Previously reported TQX and PQZ series.

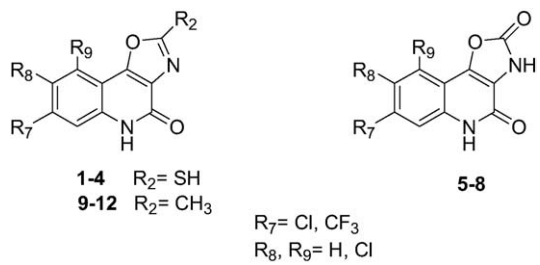


Fig. 2. Currently reported oxazolo[4,5-c]quinolin-4-one derivatives.

2. Chemistry

Compounds **1–12** (Fig. 2) were prepared using as key intermediates the 4-hydroxy-3-nitroquinolin-2(1H)ones **19–21** and **27** which were synthesized as described in Figs. 3 and 4, respectively. The 7-chloro-4-hydroxy-3-nitroquinolin-2(1H)-one **19** was obtained, differently from the reported procedure [20], by heating at 50 °C in anhydrous tetrahydrofuran the 7-chloro-1,2-dihydro-3,1-benzoxazine-2,4-dione **13** [21] and ethyl nitroacetate. The synthesis of the 5,7-dichloro-4-hydroxy-3-nitroquinolin-2(1H)-one **20** was performed as described in ref. 20, i.e. by allowing the 5,7-dichloro-4-hydroxyquinolin-2(1H)-one **17** [22] to react with 70% HNO_3 in glacial acetic acid at room temperature. The 6,7-dichloro-4-hydroxy-3-nitroquinolin-2(1H)-one **21** was obtained differently from the previously described method [20], which in our hands was inconvenient. Derivative **21** was synthesized starting from the 6,7-dichloro-1,2-dihydro-3,1-benzoxazine-2,4-dione **14,** which ensued from treatment of **13** with sulfuryl chloride, as previously reported [23]. By heating derivative **14** and NaOH in absolute methanol at 80 °C, the methyl

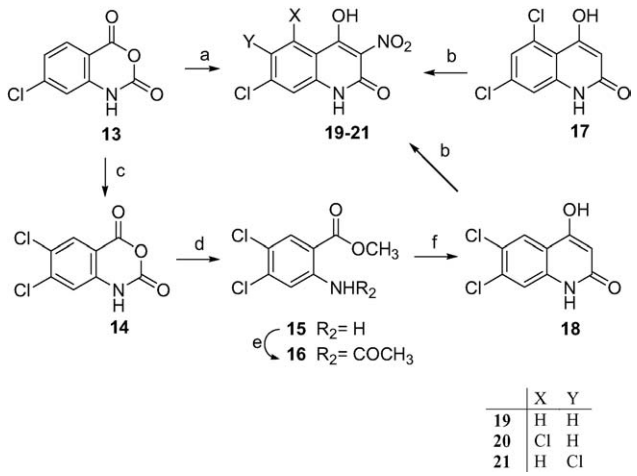


Fig. 3. Synthesis of 4-hydroxy-3-nitroquinolin-2(1H)-ones **19–21**. (a) NO_2CH_2COOEt, THF, NEt_3; (b) 70% HNO_3, glacial AcOH; (c) SO_2Cl_2, glacial AcOH, I_2; (d) anhydrous MeOH, NaOH; (e) Ac_2O, dioxane; (f) (i) $[(CH_3)_3Si]_2NK$, anhydrous THF; (ii) 6 N HCl.

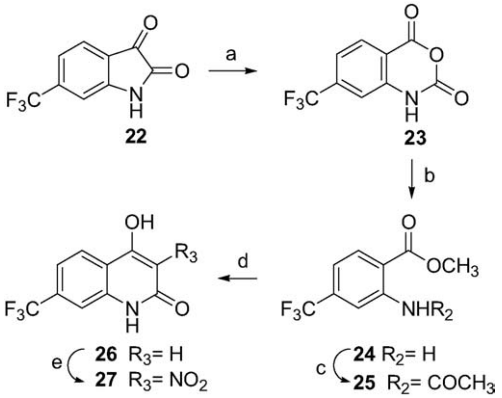


Fig. 4. Synthesis of 7-trifluoromethyl-4-hydroxy-3-nitroquinolin-2(1H)-ones **27**. (a) CrO_3, glacial AcOH, Ac_2O; (b) anhydrous MeOH, NaOH; (c) Ac_2O, dioxane; (d) (i) $[(CH_3)_3Si]_2NK$, anhydrous THF; (ii) 6 N HCl; (e) 70% HNO_3, glacial AcOH.

2-amino-4,5-dichlorobenzoate **15** was obtained which was acetylated to yield the 2-acetamido derivative **16**. This last was cyclized with potassium bis(trimethylsilyl)amide in anhydrous tetrahydrofuran to afford the 6,7-dichloro-4-hydroxyquinolin-2(1H)-one **18** [22], which was nitrated to yield the 3-nitro derivatives **21** [20]. The 7-trifluoromethyl-4-hydroxy-3-nitroquinolin-2(1H)-one **27** (Fig. 4) was obtained following the pathway above described for the preparation of **21**. Oxidation with cromic anhydride of the 6-trifluoromethylisatin **22** [24] yielded the 6-trifluoromethyl-1,2-dihydro-3,1-benzoxazine-2,4-dione **23** which was transformed into the methyl 2-amino-4-trifluoromethylbenzoate **24** [25]. Acetylation of **24** afforded the 2-acetamido derivative **25,** which was cyclized to the 7-trifluoromethyl-4-hydroxyquinolin-2(1H)-one **26**. Treatment of **26** with nitric acid gave the corresponding 3-nitro derivative **27**. The 4-hydroxy-3-nitroquinolin-(1H)-one derivatives **19–21, 27** were reduced with sodium dithionite to the corresponding

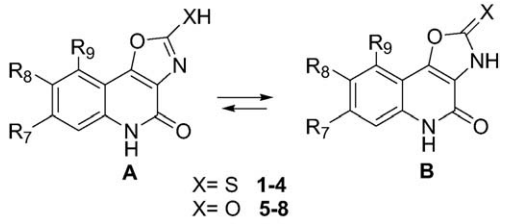
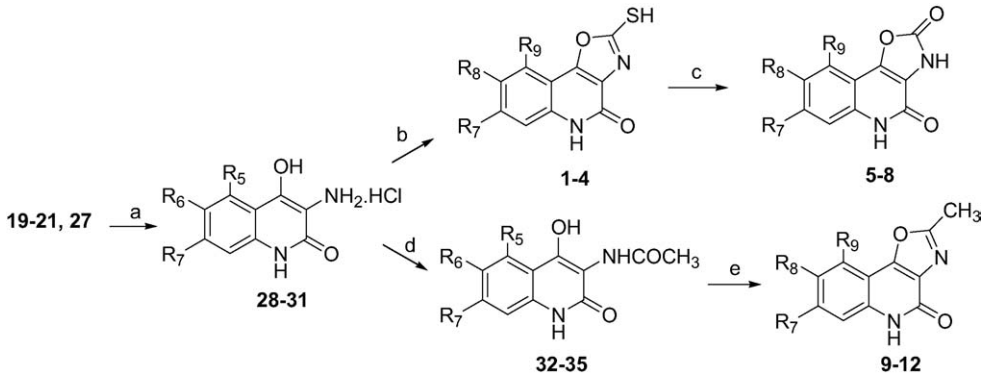


Fig. 6. Tautomers **A** and **B** of derivatives **1–8**.

3-amino-4-hydroxyquinolin-2(1H)-ones, which were isolated as hydrochlorides **28–31** (Fig. 5). These compounds are very unstable for their photosensibility and transform into coloured compounds. Thus, they cannot be recrystallized and must be handled and stored sheltered from light. However, the crude **28–31** were pure enough to be used without further purifications. Reflux of a hydroalcoholic solution of **28–31** and carbon disulfide, in the presence of KOH, gave the desidered 4,5-dihydro-2-mercapto-oxazolo[4,5-c]quinolin-4-one derivatives **1–4**. Oxidation of compounds **1–4** with hydrogen peroxide in aqueous NaOH solution furnished the 2,3,4,5-tetrahydro-oxazolo[4,5-c]quinoline-2,4-diones **5–8**.

Reaction of **28–30** with acetyl chloride afforded the 3-acetamido derivatives **32–34** which were cyclized in refluxing acetic anhydride to the 4,5-dihydro-2-methyl-oxazolo[4,5-c]quinolin-4-one derivatives **9–11**. Treatment of the 7-trifluoromethyl derivative **31** with acetyl chloride gave a complex mixture made up by of desidered 4-acetamido compound **35** and poly-acetylated derivatives (from ^{1}H-NMR analysis of the crude product). By refluxing the crude mixture with acetic anhydride, the 2-methyl-7-trifluoromethyl-oxazolo[4,5-c]quinolin-4-one **12** was obtained with satisfactory yield. Derivative **35** was easily isolated from the crude product due to its insolubility in boiling ethanol.

It has to be noted that derivatives **1–4** and **5–8** may exist in either one of the two tautomeric forms **A** and **B** (Fig. 6). As



	R_5/R_9	R_6/R_8	R_7
1, 5, 9, 28, 32	H	H	Cl
2, 6, 10, 29, 33	Cl	H	Cl
3, 7, 11, 30, 34	H	Cl	Cl
4, 8, 12, 31, 35	H	H	CF_3

Fig. 5. Synthesis of oxazolo[4,5-c]quinolin-4-ones **1–12**. (a) (i) $Na_2S_2O_4$, NaOH; (ii) 6 N HCl; (b) (i) CS_2, EtOH/H_2O, KOH; (ii) 6 N HCl; (c) 35% H_2O_2, NaOH; (d) CH_3COCl, NEt_3, THF; (e) Ac_2O.

regards compounds **1–4**, neither the ^{1}H-NMR nor IR spectral data indicated which of the two forms exists, respectively, in DMSO solution or in the solid state. Similarly, no information can be obtained from the ^{1}H-NMR data of derivatives **5–8**. In contrast, their IR spectra showed two strong bands between 1700 and 1780 cm^{-1} thus indicating that derivatives **5–8** exist in the solid state in the 2,4-dione form (tautomer **B**). To know which were the most stable tautomers, ab initio quantum chemical computational calculation has been performed on derivatives **1–8**. By using the program GAMESS [26] the energy in vacuo of both forms **A** and **B** have been calculated. The results of these studies, reported in Table 1, clearly indicate that for compounds **1–4**, the 2-mercapto forms **A** are more stable than the 2-thioxo forms **B**, having 26–30 kcal mol^{-1} lower energy than the latter. Conversely, for compounds **5–8**, tautomers **B**, i.e. the 2-oxo forms, are significantly more stable than tautomers **A**, the difference in energy being 63–66 kcal mol^{-1}. These latter results are also in accordance with the IR data of **5–8**.

Table 1
Energies of tautomers **A** and **B** of **1–8** and differences in energy between **A** and **B** (ΔE **A** – **B**)

Compound number	E^a **A**	E^a **B**	ΔE^b **A** – **B**
1	–2977.029105	–2976.987183	–26.3
2	–3890.058786	–3890.013292	–28.5
3	–3890.065003	–3890.022856	–26.4
4	–2730.473749	–2730.426285	–29.8
5	–2334.851725	–2334.957335	66.3
6	–3247.878306	–3247.980077	63.9
7	–3247.886252	–3247.990539	65.4
8	–2088.297168	–2088.401441	65.4

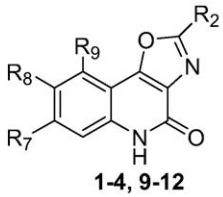
[a] Energies values are expressed in Hartree (Hartree × 627.5 = kcal mol^{-1}).
[b] ΔE values are expressed in kcal mol^{-1}.

3. Results and discussion

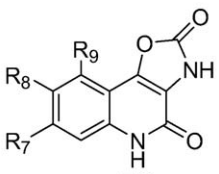
As stated above, computational studies indicated that the most stable tautomers for compounds **1–4** and **5–8** are, respectively, the 2-mercapto- and the 2-oxo-forms. However, it is well-known that the most stable structure of a molecule drawn from spectroscopical or theoretical studies may not be the most stable in the biological environment. In addition, the receptor–ligand interaction itself can stabilize a molecule in a high energy state. Nevertheless, as a great difference in energy was found between the two tautomeric forms of derivatives **1–8**, we can assume that the most stable tautomers are also the pharmacologically active ones.

Compounds **1–12** were tested for their ability to displace [^{3}H]glycine, [^{3}H]AMPA and [^{3}H]kainate from their specific sites in rat cerebral cortex membranes. The binding data are shown in Table 2 together with those of the 2-carboxylic acids **7-Cl-TQX** and **8-Cl-PQZ** and of **DCKA** (5,7-dichloro-

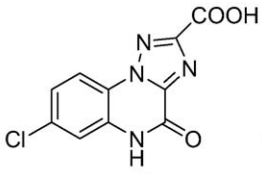
Table 2
[^{3}H]Glycine, [^{3}H]AMPA and [^{3}H]KA binding assays



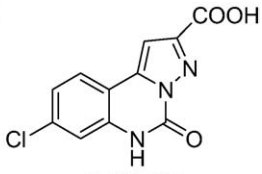
1-4, 9-12



5-8



7-Cl-TQX

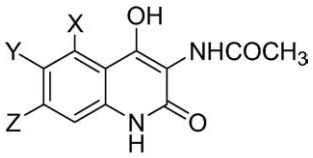


8-Cl-PQZ

					K_i (μM)[a] or I%[b]		IC_{50} (μM)[c] or I%[b]
	R_2	**R_7**	**R_8**	**R_9**	**Gly**	**AMPA**	**KA**
1	SH	Cl	H	H	0.083 ± 0.006	4.2 ± 1.4	42 ± 17
2	SH	Cl	H	Cl	0.2 ± 0.07	1.7 ± 0.1	7.4 ± 1.4
3	SH	Cl	Cl	H	0.10 ± 0.02	4.2 ± 0.4	27 ± 1.1
4	SH	CF_3	H	H	0.28 ± 0.04	2.7 ± 0.4	32 ± 4
5		Cl	H	H	3.4 ± 0.5	27%	0%
6		Cl	H	Cl	51 ± 7.0	25 ± 4	0%
7		Cl	Cl	H	1.2 ± 0.2	45%	20%
8		CF_3	H	H	63 ± 8	10%	9%
9	CH_3	Cl	H	H	46%	52 ± 8	10%
10	CH_3	Cl	H	Cl	25%	35%	0%
11	CH_3	Cl	Cl	H	25%	25%	0%
12	CH_3	CF_3	H	H	48%	87 ± 10	0%
7-Cl-TQX[d]					0.17 ± 0.02	0.78 ± 0.15	NT[e]
8-Cl-PQZ[f]					0.48 ± 0.04	2.3 ± 0.4	NT[e]
DCKA					0.09 ± 0.02	5%	8%

[a] K_i values are mean ± S.E.M. of three or four separate determinations in triplicate.
[b] Percentage of inhibition (I%) of specific binding at 100 μM concentration.
[c] IC_{50} values are mean ± S.E.M. of three or four separate determinations in triplicate.
[d] Ref. [13].
[e] Not Tested.
[f] Ref. [17].

Table 3
[^{3}H]Glycine, [^{3}H]AMPA and [^{3}H]KA binding assays



32-35

				K_i (μM)[a] or I%[b]		I%[b]
	X	**Y**	**Z**	**Gly**	**AMPA**	**KA**
32	H	H	Cl	63 ± 9	8%	0%
33	Cl	H	Cl	90 ± 8	17%	0%
34	H	Cl	Cl	12.5 ± 1.9	18%	20%
35	H	H	CF_3	40%	6%	0%

[a] K_i values are mean ± S.E.M. of three or four separate determinations in triplicate.
[b] Percentage of inhibition (I%) of specific binding at 100 μM concentration.

kynurenic acid), included as reference compounds. The intermediates **32–35** were also tested in the above cited assays (Table 3) since they belong to the class of the 4-hydroxyquinolin-2(1H)-ones thus they were likely to possess some Gly/NMDA receptor affinity.

The binding results indicate that the 2-mercapto-oxazoloquinolin-4-one derivatives **1–4** are endowed with good Gly/NMDA affinity, comparable to that of **DCKA**. Moreover, compounds **1–4** display similar Gly/NMDA affinities with respect to the corresponding TQX and PQZ derivatives [13,17], but are more selective towards this receptor. The most active and selective oxazoloquinoline derivative is the 7-chloro-substituted compound **1** (K_i = 0.083 μM) which is about 50- and 500-fold less active at the AMPA and KA receptors, respectively, than at the Gly/NMDA one. The 7,9-dichloro- derivative **2** and the 7,8-dichloro-derivative **3** also display similar good Gly/NMDA affinities. Nevertheless, while **3** is quite selective (42- and 270-fold towards AMPA and KA receptor, respectively), compound **2** is only 8.5-fold less active at the AMPA receptor than at the Gly/NMDA site, while displaying a 37-fold selectivity with respect to the KA receptor. Replacement of the 7-chloro substituent of compound **1** with a trifluoromethyl group afforded derivative **4** which shows, compared to **1**, a decreased Gly/NMDA selectivity vs. either AMPA or KA receptors. The comparable Gly/NMDA receptor affinities of the 2-mercapto-oxazoloquinoline derivatives **1–4** and their analogue TQX and PQZ 2-carboxylic acids [13,17], together with the close structural similarities of the three series, would suggest a similar binding mode of compounds **1–4** and of TQX or PQZ derivatives to the glycine receptor site. Therefore, the newly probed 2-mercapto substituent seems to replace well the previously investigated 2-carboxyl group in a hypothetical hydrogen bond with a receptor subsite.

The important role of the 2-mercapto substituent emerged when it was replaced with the 2-oxo group. This structural modification drastically decreased the Gly/NMDA affinity. In fact, compounds **5–8** are from 12- to 255-fold less active than the corresponding compounds **1–4**. It has to be noted that, differently from the 2-mercapto substituent, the 2-oxo group of **5–8** is only able to act as a hydrogen bond acceptor. Furthermore, the significantly different Gly/NMDA receptor affinity of compounds **1–4**, with respect to derivatives **5–8**, may also be associated with the replacement of the 3-nitrogen atom with the 3-NH substituent and, consequently, with the different strength of interaction of these two diverse moieties with the cationic hydrogen bond donor–receptor site. This site is reported to be a protonated arginine residue (R523), which provides an essential contribution to the binding of both agonists and antagonists of the Gly/NMDA receptor [27]. Recently, docking studies of TQX and PQZ series to a homology-based model of the Gly/NMDA receptor [28] have pointed out a bidentate interaction of R523 with both the N-3 and the adjacent carbonyl group of TQX and PQZ derivatives. Consequently, we also presumed a similar interaction for the herein reported oxazoloquinolin-4-one derivatives. Therefore, in order to explain the different Gly/NMDA affinities of the 2-mercapto derivatives **1–4** with respect to the 2-oxo compounds **5–8,** we performed an ab initio computational study to calculate the different interaction strength of the 3-nitrogen-4-C=O moiety of **1–8** with the *N*-methylguanidinium ion (probe **I**) which was used to model the protonated side-chain of the arginine residue. In addition, since we hypothesized that the 2-substituents of **1–4** and **5–8**, SH and C=O, respectively, were directly involved in the anchoring to the receptor site by forming a hydrogen bond, we also evaluated the different interaction strengths of these groups with MeOH (probe **II**), chosen because it can act as either hydrogen bond donor or acceptor. The computational calculations were performed on the 7-chloro-2-mercapto-oxazoloquinolin-4-one **1** and on the corresponding 2-oxo compound **5**. To evaluate the contribution energy of each probe to the total interaction energy, we considered one probe at a time. Thus, probes **I** and **II** were placed, respectively, in the neighborhood of the 3,4- and 1,2-positions of compounds **1** and **5**. The four complexes were minimized with GAMESS and their structures reported in Figs. 7 and 8. The interaction energies of derivatives **1** and **5** with each probe are shown in Table 4. As can be seen from the energy values, the strength of interaction of the 2-mercapto derivative **1** with probe **I** ($\Delta E_{\text{probe I}}$ = –37.94 kcal mol^{-1}) is higher than that of the 2-oxo compound **5** ($\Delta E_{\text{probe I}}$ = –25.20 kcal mol^{-1}), in accordance with the higher affinity of **1** with respect to **5**. The energy difference resulted from the different binding modes of this probe with the two compounds (Fig. 7). Indeed, the *N*-methylguanidinium ion forms a bidentate interaction with both the 3-nitrogen and the 4-carbonyl oxygen of the 2-mercapto derivative **1**, while its interaction with compound **5** involves only the 4-carbonyl oxygen, due to the presence of the NH group at the 3-position.

Interaction of MeOH (probe **II**) with the 2-substituent of compounds **1** and **5** occurs in two different modes (Fig. 8), as the 2-mercapto group and the 2-oxo are, respectively, a hydrogen bond donor and acceptor. A difference of 2.37 kcal mol^{-1}

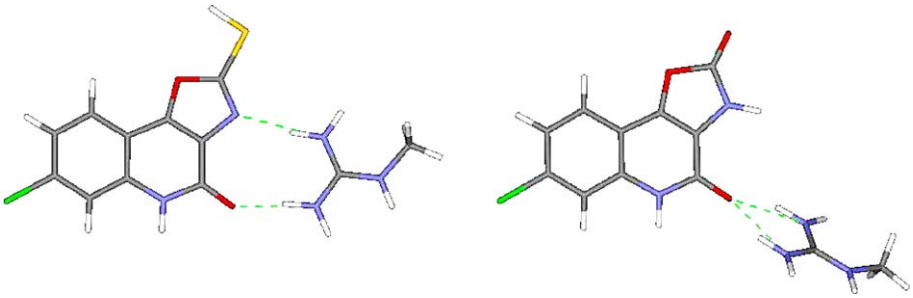


Fig. 7. Optimized geometries of the complexes between *N*-methylguanidinium ion (probe **I**) and compounds **1** (on the left) and **5** (on the right).

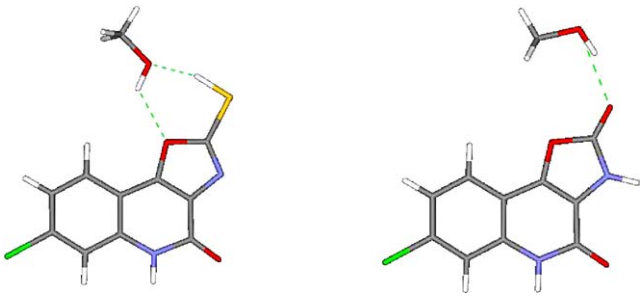


Fig. 8. Optimized geometries of the complexes between MeOH (probe **II**) and compounds **1** (on the left) and **5** (on the right).

Table 4
Interaction energies (ΔE) of compounds **1**, **5** and **9** with *N*-methylguanidinium ion (probe **I**) and MeOH (probe **II**)

	$\Delta E_{\text{probe I}}$ [a]	$\Delta E_{\text{probe II}}$ [a]
1	–37.94	–9.91
5	–25.20	–7.54
9	–38.53	nc[b]

[a] The interaction energies values, expressed in kcal mol^{-1}, were calculated as follows: $\Delta E = E_{\text{complex}} - (E_{\text{compound}} + E_{\text{probe}})$.

[b] Not calculated due to the incapability of the methyl group to form a hydrogen bond.

was found, as the hydrogen bond engaged with compound **1** ($\Delta E_{\text{probe II}} = -9.91$ kcal mol^{-1}) is slightly stronger. Therefore, on the basis of these quantum chemical calculations, we can assume that the significantly higher affinities of the 2-mercapto derivatives **1–4**, with respect to the corresponding 2-oxo derivatives **5–8**, are above all due to the stronger hydrogen bond with the arginine receptor site. This stronger interaction, in its turn, is due to the presence of the 3-sp^2-nitrogen atom, which can reinforce the hydrogen bond that the 4-carbonyl oxygen forms with the arginine residue.

Obviously, the calculated interaction energy values are not binding energy, therefore, they can only be used to compare the relative stabilities of the complexes. Thus, although the interaction energies of derivatives **1** and **5** with probe **I** are significantly higher than those resulting from interaction with probe **II**, the 3-nitrogen-4-CO moiety may not be the only one to play a pivotal role in the anchoring to the receptor site. On the contrary, the binding data of the 2-methyl-derivatives **9–12** point out the importance of a suitable substituent at the 2-position. In fact, although these derivatives might be hypothesized to bind efficaciously to the arginine site, they are completely inactive at the Gly/NMDA receptor. Quantum chemical calculations carried out on the 2-methyl-7-chloro-derivative **9** show that interaction energy with probe **I** ($\Delta E_{\text{probeI}} = -38.53$ kcal mol^{-1}) is as high as that of the corresponding 2-mercapto derivative **1**. Thus, the complete lack of affinity of **9–12** has to be attributed to the incapability of the 2-methyl group to give a hydrogen bond interaction with the receptor site. These data point out that in this class of compounds the presence of a 3-sp^2-nitrogen atom is an important but not the only requirement since a 2-substituent able to form a hydrogen bond, with a putative hydrogen bond donor or acceptor, is also necessary to permit an efficacious binding to the Gly/NMDA receptor site.

Both these structural features were also essential for AMPA and KA receptor recognition. Indeed, in contrast to compounds **1–4,** which bind to these receptors with micromolar affinities, derivatives **5–8** and **9–12** are inactive both at AMPA and KA receptors, with the exceptions of compounds **6**, **9** and **12,** which show AMPA affinities in the high micromolar range. However, as previously stated, some 8-heteroaryl-oxazolo[4,5-c]quinoline-2,4-dione derivatives have already been described as AMPA antagonists [18]. Thus, at first glance, the very low AMPA affinity of our oxazoloquinoline-2,4-dione derivatives **5–8** could be quite unexpected. Actually, inactivity of **5–8** has to be ascribed to the lack of the 8-heteroaryl moiety. In fact, such a substituent is known to enhance AMPA affinity of different classes of antagonists [14–17,19] and, accordingly, it was revealed to be of paramount importance in the oxazolo[4,5-c]quinoline-2,4-dione series.

As expected, the 3-acetamido-4-hydroxyquinolin-2-(1H)-ones **32–34**, bearing chloro-substituent(s) on the benzofused ring, display micromolar Gly/NMDA affinities while the 7-trifluoromethyl- derivative **35** is much less active. Compounds **32–35** are completely devoid of both AMPA and KA receptor affinities.

The selected compounds **1, 3–4** were tested to assess their antagonistic activity at the Gly/NMDA receptor by evaluating their ability to inhibit the stimulated [^{3}H]-(+)-MK-801 ((+)-5-methyl-10,11-dihydro-5H-benzo[a,d]cyclohepten-5,10-imine) binding [29–31]. The binding results, reported in Table 5, indicate that derivatives **1, 3–4** are antagonists with potencies similar to that of **DCKA,** included as reference antagonist. Compounds **1, 3–4** and **DCKA** were also evaluated for their ability to inhibit depolarization induced by AMPA or NMDA in mouse cortical wedge preparations

Table 5
Inhibition of stimulated [^{3}H] (+)MK-801 binding and functional antagonism at NMDA and AMPA sites of some selected derivatives

	IC_{50} (μM)[a]	IC_{50} (μM)[b]	
	[^{3}H] (+)MK-801	NMDA	AMPA
1	1.5 ± 0.2	0.84 ± 0.1	4.2 ± 0.5
3	0.9 ± 0.1	1.7 ± 0.2	13 ± 2
4	1.1 ± 0.15	0.3 ± 0.05	1.5 ± 0.2
DCKA	0.74 ± 0.09	4.7 ± 0.9	52 ± 11

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

[b] Concentration necessary for 50% inhibition (IC_{50}) of depolarization induced by S-AMPA or NMDA in mouse cortical wedge preparation. IC_{50} values are mean ± S.E.M. of three separate determinations.

(Table 5). All the tested compounds inhibited AMPA and NMDA responses in a reversible manner. The potencies of derivatives **1**, **3–4** are in agreement with their binding affinities since they show higher inhibitory potencies on NMDA-evoked response than on AMPA-induced depolarization. Moreover, **1**, **3–4** showed, on the whole, higher inhibitory potencies than **DCKA** at the NMDA receptor.

4. Conclusion

The present study has produced new oxazolo[4,5-c]quinolin-4-one derivatives, which are endowed with a good affinity and selectivity toward the Gly/NMDA receptor (compounds **1–4**). SAR studies have pointed out the necessary structural requirements of these tricyclic compounds to achieve a good receptor–ligand interaction. In particular, as a new result, the profitable effect of the 2-mercapto group has been determined. The significantly higher affinity of the 2-mercapto derivatives **1–4** with respect to the 2-oxo derivatives **5–8** has been explained on the basis of ab initio quantum chemical calculations which showed the importance of a 3-sp^2-nitrogen atom which, together with the 4-carbonyl oxygen, is likely to be involved in a hydrogen bond with the hydrogen- bond donor arginine site of the Gly/NMDA receptor. Nevertheless, the complete inactivity of the 2-methyl derivatives **9–12** indicates that the presence of the 3-sp^2-nitrogen atom is not the only requirement for a good affinity, but a 2-substituent able to form a hydrogen bond is also necessary.

5. Experimental protocols

5.1. 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 S for derivatives **1–4**) and the results were within ± 0.4% of the theoretical, unless otherwise stated. The IR spectra were recorded with a Perkin-Elmer Spectrum RX I spectrometer in Nujol mulls and are expressed in cm^{-1}. The ^{1}H-NMR spectra were obtained with a Varian Gemini 200. The chemical shifts are reported in δ (ppm) and are relative to the central peak of the solvent that is always DMSO-d_6.

5.1.1. 7-Chloro-4-hydroxy-3-nitroquinolin-2(1H)-one (**19**) [20]

The 7-chloro-1,2-dihydro-3,1-benzoxazine-2,4-dione (**13**) [21] (5 g, 25.3 mmol) was added to a solution of ethyl nitroacetate (4.2 ml, 37.9 mmol) and anhydrous NEt_3 (5.3 ml, 37.9 mmol) in anhydrous THF (100 ml). The mixture was heated at 55 °C for 27 h. Evaporation of the solvent at reduced pressure yielded a turry residue, which was treated with Et_2O (50 ml). The ethereal phase was decanted and this treatment was repeated twice. Finally, the crude residue was taken up with EtOAc (5 ml) and scratched to give a suspension, which was kept overnight in a refrigerator. The solid was filtered, washed with Et_2O and dissolved in H_2O (50 ml). Acidification of the solution with 6 N HCl afforded a solid, which was collected by filtration and washed with water. Yield: 35%; m.p. 221–223 °C dec (EtOH) (lit. 220–222 °C [20]); ^{1}H-NMR 7.23–7.29 (m, 2H, H_5 + H_8), 7.97 (dd, 1H, H_6, J = 8.8 Hz, 4.4 Hz), 11.88 (s, 1H, exchangeable with D_2O); IR 1368, 1523, 1676, 3190.

5.1.2. 5,7-Dichloro-4-hydroxy-3-nitroquinolin-2(1H)-one (**20**) [20]

Seventy percent HNO_3 (5.2 ml) was added to a suspension of 5,7-dichloro-4-hydroxyquinolin-2(1H)-one (**17**) [22] (4 g, 17.4 mmol) in glacial AcOH (24 ml). The mixture was heated at 90 °C for 2 h, then cooled at room temperature. The solid was collected by filtration, abundantly washed with water and then with Et_2O. Yield: 82%; m.p. 198–200 °C dec (EtOH) (lit. 170–171 °C [20]); ^{1}H-NMR 7.18–7.24 (m, 2H, ar), 11.48 (s, 1H, exchangeable with D_2O); IR 1353, 1525, 1670.

5.1.3. 6,7-Dichloro-1,2-dihydro-3,1-benzoxazine-2,4-dione (**14**) [23]

A suspension of derivative **13** (5 g, 25.3 mmol) in SO_2Cl_2 (165 ml) and glacial AcOH (45 ml), in the presence of catalytic amount of I_2, was heated at 80 °C for 65 h. Evaporation of the solvent at reduced pressure afforded a solid which was suspended in H_2O (30 ml) and filtered. Yield: 80%; m.p. 295–297 °C (EtOH); ^{1}H-NMR 7.29 (s, 1H, ar), 8.08 (s, 1H, ar), 11.95 (s, 1H, NH).

5.1.4. Methyl 2-amino-4,5-dichlorobenzoate (**15**)

Compound **14** (12.7 mmol) was suspended in an anhydrous methanolic 0.1 M NaOH solution (60 ml). The mixture was heated at 80 °C for a few minutes, until it became a brownish solution. After cooling at room temperature and dilution with H_2O, a solid precipitated which was collected and washed with H_2O. Yield: 85%; m.p. 119–121 °C (MeOH);

^{1}H-NMR 3.79 (s, 3H, CH_3), 6.89 (s, 2H, NH_2), 7.04 (s, 1H, ar), 7.78 (s, 1H, ar); IR 1703, 3375, 3491. 'Anal. $C_8H_7Cl_2NO_2$ (C, H, N)'.

*5.1.5. Methyl 2-acetamido-4,5-dichlorobenzoate (**16**)*

A solution of **15** (4.5 mmol) in Ac_2O (5 ml) and anhydrous dioxane (8 ml) was heated at 50 °C, under nitrogen atmosphere, for 13 h. The mixture was diluted with H_2O (2.5 ml) and stirred for about 15 min, then the solvent was evaporated at reduced pressure to give an oil, which slowly solidified. Yield: 75%; m.p. 122–124 °C (MeOH); ^{1}H-NMR 2.13 (s, 3H, CH_3 amide), 3.85 (s, 3H, CH_3), 8.00 (s, 1H, ar), 8.49 (s, 1H, ar), 10.54 (s, 1H, NH); IR 1698, 1716, 3305. 'Anal.$C_{10}H_9ClNO_3$ (C, H, N)'.

*5.1.6. 6,7-Dichloro-4-hydroxy-quinolin-2(1H)-ones (**18**) [22]*

A solution of compounds **16** (7.6 mmol) in anhydrous THF (30 ml) was added dropwise to a 0.5 M solution of potassium bis(trimethylsilyl)amide (72.5 ml, 36.3 mmol) in toluene at –78 °C under nitrogen atmosphere. After the addition was completed, the mixture was kept at –78 °C for about 1 h, then it was left to return to room temperature. The mixture was quenched with ice H_2O (150 ml) and stirred for 15 min. The aqueous layer was washed with EtOAc (15 ml × 3) and then acidified with 6 N HCl. The solid was collected, abundantly washed with H_2O and dried. Yield: 68%; m.p. > 300 °C (DMF) (lit. 335 °C [22]); ^{1}H-NMR 5.73 (s, 1H, H-3), 7.42 (s, 1H, ar), 7.85 (s, 1H, ar), 11.37 (s, 1H, exchangeable with D_2O), 11.65 (s, 1H, exchangeable with D_2O); IR 1689, 3621.

*5.1.7. 6,7-Dichloro-4-hydroxy-3-nitroquinolin-2(1H)-ones (**21**) [20]*

Compound **18** was nitrated following the experimental conditions described above to prepare **20** from **17**, with the only difference being the reaction time that, for **18,** was shorter (about 10 min). Yield: 74%; m.p. 170–171 °C (EtOH) (lit. 235–236 °C [20]); ^{1}H-NMR 7.40 (s, 1H, ar), 8.08 (s, 1H, ar), 11.49 (s, 1H, exchangeable with D_2O); IR 1377, 1528, 1664, 3162, 3400. 'Anal.$C_9H_4Cl_2N_2O_4$ (C, H, N)'.

*5.1.8. 7-Trifluoromethyl-1,2-dihydro-3,1-benzoxazine-2,4-dione (**23**)*

CrO_3 (3.6 g, 36.7 mmol) was added portionwise to a hot (90 °C) suspension of the 6-trifluoromethylisatin (**22**) [24] (5 g, 21.6 mmol) in glacial AcOH (20 ml) and Ac_2O (20 ml). The mixture was heated at 90 °C for 2 h. After cooling at room temperature, the suspension was diluted with water (50 ml) and the solid collected and abundantly washed with H_2O. Yield: 86%; m.p. 240–242 °C (EtOAc); ^{1}H-NMR 7.37 (s, 1H, ar), 7.54 (d, 1H, ar, J = 8.4 Hz), 8.10 (d, 1H, ar, J = 8.4 Hz), 11.95 (s, 1H, NH). 'Anal.$C_9H_4F_3NO_3$ (C, H, N)'.

*5.1.9. Methyl 2-amino-4-trifluoromethylbenzoate (**24**) [25]*

The title compound was obtained from **23** as described above for the preparation of **15**. Yield: 80%; m.p. 65–68 °C (H_2O/EtOH) (lit. 60–62 °C [25]); ^{1}H-NMR 3.80 (s, 3H, CH_3), 6.76 (d, 1H, ar, J = 8.4 Hz), 6.96 (s, 2H, NH_2), 7.12 (s, 1H, H-3), 7.85 (d, 1H, J = 8.4 Hz); IR 1687, 3368, 3398, 3480.

*5.1.10. Methyl 2-acetamido-4-trifluoromethylbenzoate (**25**)*

The title compound was obtained from **24** as described above for the preparation of **16**. Yield: 94%; m.p. 88–90 °C (petroleum ether 30–60°); ^{1}H-NMR 2.12 (s, 3H, CH_3 amide), 3.86 (s, 3H, CH_3), 7.51 (d, 1H, ar, J = 8.2 Hz), 8.04 (d, 1H, ar, J = 8.2 Hz), 8.50 (s, 1H, H-3), 10.58 (s, 1H, NH); IR 1590, 1701, 3263. 'Anal.$C_{11}H_{10}F_3NO_3$ (C, H, N)'.

*5.1.11. 7-Trifluoromethyl-4-hydroxyquinolin-2(1H)-one (**26**)*

The title compound was prepared from **25** as described above for the synthesis of **18**. Yield: 60%; m.p. > 300 °C (2-Methoxyethanol); ^{1}H-NMR 5.82 (s, 1H, H-3), 7.42 (d, 1H, ar, J = 8.4 Hz), 7.55 (s, 1H, ar), 7.95 (d, 1H, ar, J = 8.4 Hz), 11.45 (s, 1H, exchangeable with D_2O), 11.67 (br s, 1H, exchangeable with D_2O); IR 1586, 1610, 3400. 'Anal. $C_{10}H_6F_3NO_2$ (C, H, N)'.

*5.1.12. 7-Trifluoromethyl-4-hydroxy-3-nitroquinolin-2(1H)-one (**27**)*

The title compounds was prepared from **26** as described above for the synthesis of **21**. Yield: 82%; m.p. 182–183 °C (EtOAc); ^{1}H-NMR 7.53 (d, 1H, ar, J = 7.8 Hz), 7.60 (s, 1H, H-8), 8.20 (d, 1H, ar, J = 7.8 Hz), 11.92 (s, 1H, exchangeable with D_2O); IR 1334, 1558, 1668, 1684, 3167. 'Anal. $C_{10}H_5F_3N_2O_4$ (C, H, N)'.

*5.1.13. General procedure for the synthesis of 3-amino-4-hydroxyquinolin-2(1H)-one hydrochlorides **28–31***

$Na_2S_2O_4$ (2.1 g, 11.9 mmol) was added to a 1 M NaOH aqueous solution (20 ml) of the 3-nitro derivatives **19–21, 27** (2.1 mmol). The mixture was sheltered from light and stirred for 30 min. After cooling at 0 °C, the solution was acidified with 6 N HCl and the solid which precipitated was quickly collected by filtration, washed with a few drops of cold water and subsequently with petroleum ether and then dried in vacuo. The crude compounds **28–31**, obtained in almost quantitative yields, were photosensitive, thus they could not be recrystallized and had to be handled and stored sheltered from light. Nevertheless, they were pure enough to be used in the next step.

*5.1.13.1. 3-Amino-7-chloro-4-hydroxyquinolin-2(1H)-one hydrochloride (**28**).* ^{1}H-NMR 5.00 (br s, 3H, NH_3^+), 7.11(d 1H, ar J = 8.4 Hz), 7.24 (s, 1H, ar), 7.67 (dd, 1H, ar, J = 8.4 Hz, J = 2.2 Hz), 11.62 (br s 1H, exchangeable with D_2O).

*5.1.13.2. 3-Amino-5,7-dichloro-4-hydroxyquinolin-2(1H)-one hydrochloride (**29**).* ^{1}H-NMR 6.20 (br s, 3H, NH_3^+), 7.16 (s, 1H, ar), 7.22 (s, 1H, ar), 11.80 (br s, 1H, exchangeable with D_2O).

*5.1.13.3. 3-Amino-6,7-dichloro-4-hydroxyquinolin-2(1H)-one hydrochloride (**30**).* ^{1}H-NMR 5.80 (br s, 3H, NH_3^+), 7.37 (s, 1H, ar), 7.78 (s, 1H, ar), 11.73 (br s 1H, exchangeable with D_2O).

*5.1.13.4. 3-Amino-7-trifluoromethyl-4-hydroxyquinolin-2(1H)-one hydrochloride (**31**).* ^{1}H-NMR 6.30 (br s, 3H, NH_3^+), 7.41 (d, 1H, ar, J = 8.2 Hz), 7.54 (s, 1H, H-8), 7.87 (d, 1H, ar, J = 8.2 Hz), 11.84 (s, 1H, exchangeable with D_2O).

*5.1.14. General procedure for the synthesis of 4,5-dihydro-2-mercapto-oxazolo[4,5-c]quinolin-4-ones **1–4***

To a suspension of crude **28–31** (3.6 mmol) in aqueous EtOH (12 ml of EtOH and 2 ml of H_2O), KOH (0.45 g, 8.0 mmol) and CS_2 (2.2 ml, 36.4 mmol) were added. After refluxing for 5 h, the suspension was cooled at room temperature, diluted with H_2O (40 ml) and acidified with 6 N HCl. The precipitate was collected, washed with H_2O and dried.

*5.1.14.1. 7-Chloro-4,5-dihydro-2-mercapto-oxazolo[4,5-c]quinolin-4-one (**1**).* Yield: 68%; m.p. > 300 °C (EtOH); ^{1}H-NMR 7.35 (d, 1H, ar, J = 8.8 Hz), 7.46 (s, 1H, ar), 7.84 (d, 1H, ar, J = 8.8 Hz), 12.3 (br s, 1H, exchangeable with D_2O), 14.10 (br s, 1H, exchangeable with D_2O); IR 1674, 3400–3650. 'Anal. $C_{10}H_5ClN_2O_2S$ (C, H, N, S)'.

*5.1.14.2. 7,9-Dichloro-4,5-dihydro-2-mercapto-oxazolo[4,5-c]quinolin-4-one (**2**).* Yield: 56%; m.p. > 300 °C (2-Methoxyethanol); ^{1}H-NMR 7.42 (s, 1H, ar), 7.56 (s, 1H, ar), 12.51 (s, 1H, exchangeable with D_2O), 14.50 (br s,1H, exchangeable with D_2O); IR 1697. 'Anal. $C_{10}H_4Cl_2N_2O_2S$ (C, H, N, S)'.

*5.1.14.3. 7,8-Dichloro-4,5-dihydro-2-mercapto-oxazolo[4,5-c]quinolin-4-one (**3**).* Yield: 90%; m.p. > 300 °C (EtOH); ^{1}H-NMR 7.61 (s, 1H, ar), 8.10 (s, 1H, ar), 12.38 (br s, 1H, exchangeable with D_2O), 14.20 (br s, 1H, exchangeable with D_2O); IR 1700, 3383. 'Anal. $C_{10}H_4Cl_2N_2O_2S$ (C, H, N, S)'.

*5.1.14.4. 4,5-Dihydro-2-mercapto-7-trifluoromethyl-oxazolo-[4,5-c]quinolin-4-one (**4**).* Yield: 53%; m. p. > 300 °C (EtOH); ^{1}H-NMR 7.63 (d, 1H, ar, J = 8.4 Hz), 7.78 (s, 1H, H-6), 8.05 (d, 1H, ar, J = 8.4 Hz), 12.50 (s, 1H, exchangeable with D_2O), 14.75 (br s, 1H, exchangeable with D_2O); IR 1687, 1710. 'Anal. $C_{11}H_5F_3N_2O_2S$ (C, H, N, S)'.

*5.1.15. General procedure for the synthesis of 2,3,4,5-tetrahydro-oxazolo[4,5-c]quinoline-2,4-diones **5–8***

Thirty-five percent H_2O_2 (5 ml) was dropwise added to a 10% NaOH solution (22 ml) of compounds **1–4** (1.71 mmol) while maintaining the temperature at 15 °C. After the addition, the mixture was stirred at 15 °C for 30 min, then it was left to return to room temperature. The suspension was acidified to pH 2 with 6 N HCl and the solid collected and washed with H_2O.

*5.1.15.1. 7-Chloro-2,3,4,5-tetrahydro-oxazolo[4,5-c]quinoline-2,4-dione (**5**).* Yield: 84%; m.p. > 300 °C (DMF); ^{1}H-NMR 7.33 (d, 1H, ar, J = 8.6 Hz), 7.45 (s. 1H, H-6), 7.72 (d, 1H, J = 8.6 Hz), 12.18 (s, 1H, exchangeable with D_2O), 12.32 (s, 1H, exchangeable with D_2O); IR 1697, 1789, 3220. 'Anal. $C_{10}H_5ClN_2O_3$ (C, H, N)'.

*5.1.15.2. 7,9-Dichloro-2,3,4,5-tetrahydro-oxazolo[4,5-c]quinoline-2,4-dione (**6**).* Yield: 68%; m.p. > 300 °C (DMF); ^{1}H-NMR 7.40 (s, 1H, ar), 7.49 (s, 1H, ar), 12.39 (s, 1H, exchangeable with D_2O), 12.49 (s, 1H, exchangeable with D_2O); IR 1701, 1750, 3194. 'Anal. $C_{10}H_4Cl_2N_2O_3$ (C, H, N)'.

*5.1.15.3. 7,8-Dichloro-2,3,4,5-tetrahydro-oxazolo[4,5-c]quinoline-2,4-dione (**7**).* Yield: 60%; m.p. > 300 °C (EtOH); ^{1}H-NMR 7.59 (s, 1H, ar), 7.93 (s, 1H, ar), 12.25 (s, 1H, exchangeable with D_2O), 12.44 (s, 1H, exchangeable with D_2O); IR 1700, 1780, 3120. 'Anal. $C_{10}H_4Cl_2N_2O_3$ (C, H, N)'.

*5.1.15.4. 2,3,4,5-Tetrahydro-7-trifluoromethyl-oxazolo[4,5-c]quinolin-2,4-dione (**8**).* Yield: 55%; m.p. > 300 °C (DMF); ^{1}H-NMR 7.60 (d, 1H, ar, J = 8.2 Hz), 7,76 (s, 1H, H-6), 7.92 (d, 1H, ar, J = 8.2 Hz), 12.37 (s, 1H, exchangeable with D_2O), 12.50 (s, 1H, exchangeable with D_2O); IR 1700, 1780, 3230. 'Anal. $C_{11}H_5F_3N_2O_3$ (C, H, N)'.

*5.1.16. General procedure for the synthesis of 3-acetamido-4-hydroxyquinolin-2(1H)ones **32–34***

Anhydrous NEt_3 (0.59 ml, 4.25 mmol) and CH_3COCl (0.18 ml, 2.55 mmol) were dropwise added to a suspension of derivatives **28–30** (1.70 mmol) in anhydrous THF (10 ml). The mixture, sheltered from light, was refluxed for 5 h, cooled at room temperature, diluted with H_2O (20 ml) and acidified with 6 N HCl. The solid was collected and washed with water.

*5.1.16.1. 3-Acetamido-7-chloro-4-hydroxyquinolin-2(1H)one (**32**).* Yield: 98%; m.p. > 300 °C (2-Methoxyethanol); ^{1}H-NMR 2.20 (s, 3H, CH_3), 7.22 (d, 1H, ar, J = 8.8 Hz), 7.28 (s, 1H, H-8), 7.83 (d, 1H, ar, J = 8.8 Hz), 9.73(s, 1H, NHAc), 11.88 (s,1H, exchangeable with D_2O), 11.99 (br s, 1H, exchangeable with D_2O); IR 1606, 1633, 1658, 3326. 'Anal. $C_{11}H_9ClN_2O_3$ (C, H, N)'.

*5.1.16.2. 3-Acetamido-5,7-dichloro-4-hydroxyquinolin-2(1H)one (**33**).* Yield: 54%; m.p. 298 °C dec (2-Methoxyethanol); ^{1}H-NMR 2.18 (s, 3H, CH_3), 7.28 (d, 1H, ar, J = 1.8 Hz), 7.33 (d, 1H, ar, J = 1.8 Hz), 9.70 (s, 1H, NHAc), 11.68 (s, 1H, exchangeable with D_2O), 12.03 (s, 1H, exchangeable with D_2O); IR 1620, 1661, 3313. 'Anal. $C_{11}H_8Cl_2N_2O_3$ (C, H, N)'.

*5.1.16.3. 3-Acetamido-6,7-dichloro-4-hydroxyquinolin-2(1H)one (**34**).* Yield: 68%; m.p. > 300 °C (2-Methoxyethanol); ^{1}H-NMR 2.20 (s, 3H, CH_3), 7.43 (s, 1H, ar), 7.93 (s, 1H, ar), 9.78 (s, 1H, NHAc), 11.97 (s, 1H, exchangeable with D_2O), 12.11 (s, 1H, exchangeable with D_2O); IR 1603, 1629, 1661, 3329. 'Anal. $C_{11}H_8Cl_2N_2O_3$ (C, H, N)'.

*5.1.17. 3-Acetamido-4-hydroxy-7-trifluoromethylquinolin-2(1H)one (**35**)*

Anhydrous NEt_3 (0.76 ml, 5.48 mmol) and CH_3COCl (0.23 ml, 3.29 mmol) were dropwise added to a suspension of derivative **31** (2.19 mmol) in anhydrous THF (15 ml). The mixture, sheltered from light, was refluxed for 1 h 45 min, cooled at room temperature, diluted with H_2O (50 ml) and acidified with 6 N HCl. The crude solid, made up of a mixture of **35** and poly-acetylated derivatives (^{1}H-NMR analysis), was collected, washed with H_2O and used without further purification for the synthesis of derivative **12**. Pure derivative **35** was obtained by treating the crude product with boiling EtOH. The hot suspension was filtered and the solid collected. Yield: 20%; m.p. > 300 °C (2-Methoxyethanol) ^{1}H-NMR 2.25 (s, 3H, CH_3), 7.52 (d, 1H, H-6, J = 8.2 Hz), 7.60 (s, 1H, H-8), 8.06 (d, 1H, H-5, J = 8.2 Hz), 9.84 (s, 1H, exchangeable with D_2O), 12.10 (s, 2H, exchangeable with D_2O); IR 1660, 1623, 3328. 'Anal. $C_{12}H_9F_3N_2O_3$ (C, H, N)'.

*5.1.18. General procedure for the synthesis of 4,5-dihydro-2-methyl-oxazolo[4,5-c]quinolin-4-ones **9–12***

Derivatives **32–34** (2.02 mmol) or the crude mixture (0.31 g) described above in the synthesis of **35**, were refluxed in Ac_2O (4 ml) until the disappearance of the starting material (16–25 h). After cooling at room temperature, the solid was collected by filtration and washed with H_2O.

*5.1.18.1. 7-Chloro-4,5-dihydro-2-methyl-oxazolo[4,5-c]quinolin-4-one (**9**).* Yield: 80%; m.p. > 300 °C (DMF); ^{1}H-NMR 2.63 (s, 3H, CH_3), 7.31 (d, 1H, ar, J = 8.4 Hz), 7.47 (s, 1H, H-8), 7.83 (d, 1H, J = 8.4 Hz), 11.98 (s, 1H, NH); IR 1693, 3228. 'Anal. $C_{11}H_7ClN_2O_2$ (C, H, N)'.

*5.1.18.2. 7,9-Dichloro-4,5-dihydro-2-methyl-oxazolo[4,5-c]quinolin-4-one (**10**).* Yield: 90%; m.p. > 300 °C (DMF); ^{1}H-NMR 2.65 (s, 3H, CH_3), 7.44 (s, 1H, ar), 7.53 (s, 1H, ar), 12.22 (s, 1H, NH); IR 1698. 'Anal. $C_{11}H_6Cl_2N_2O_2$ (C, H, N)'.

*5.1.18.3. 7,8-Dichloro-4,5-dihydro-2-methyl-oxazolo[4,5-c]quinolin-4-one (**11**).* Yield: 85%; m.p. > 300 °C (DMF) ^{1}H-NMR 2.65 (s, 3H, CH_3), 7.64 (s, 1H, ar), 8.13 (s, 1H, ar), 12.10 (s, 1H, NH); IR 1689. 'Anal. $C_{11}H_6Cl_2N_2O_2$ (C, H, N)'.

*5.1.18.4. 7-Trifluoromethyl-4,5-dihydro-2-methyl-oxazolo[4,5-c]quinolin-4-one (**12**).* Yield: 60%; m.p. > 300 °C (DMF); ^{1}H-NMR 2.69 (s, 3H, CH_3), 7.61 (d, 1H, H-8, J = 8.3 Hz), 7.80 (s, 1H, H-6), 8.07 (d, 1H, H-9, J = 8.3 Hz), 12.21 (s, 1H, NH). IR 1687, 3356. 'Anal. $C_{12}H_7F_3N_2O_2$ (C, H, N)'.

5.2. Pharmacology

5.2.1. Binding assays

Rat cortical synaptic membrane preparation, [^{3}H]glycine, [^{3}H]AMPA and [^{3}H]-(+)-MK-801 binding experiments were performed following the procedure reported in Refs. [32,33] and [29], respectively. The high affinity [^{3}H]kainate binding assays were performed on rat cortical membrane, according to a previously described method [17].

5.2.2. Electrophysiological assays

The mouse cortical wedge preparations described by Mannaioni et al. [34] was used while the electrophysiological assays were performed following the procedures described in Ref. [17].

5.2.3. Sample preparation and result calculation

A stock 1 mM solution of the tested compound was prepared in 50% DMSO and the subsequent dilutions were accomplished in buffer. The IC_{50} values were calculated from three or four displacement curves based on four to six scalar concentrations of the tested compound in triplicate using the ALLFIT computer program [35]. K_i values were calculated according to the Cheng-Prusoff equation [36]. K_L for [^{3}H] glycine and [^{3}H]-DL-AMPA were 75 ± 6 and 28 ± 3 nM, respectively.

5.3. Quantum chemical computational calculations

Ab initio quantum chemical calculations were performed using the program package GAMESS [26]. Geometries of molecules and molecule-probe complexes were optimized in vacuo at the restricted Hartree Fock (RHF) level with the N21-3 basis set.

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