Structural Investigation of the 7-Chloro-3-hydroxy-1*H*-quinazoline-2,4-dione Scaffold to Obtain AMPA and Kainate Receptor Selective Antagonists. Synthesis, Pharmacological, and Molecular Modeling Studies

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In this paper, the study of new 7-chloro-3-hydroxy-1*H*-quinazoline-2,4-dione derivatives, designed as AMPA and kainate (KA) receptor antagonists, is reported. Some derivatives bear different carboxy-containing alkyl chains on the 3-hydroxy group, while various heterocyclic rings or amide moieties are present at the 6-position of other compounds. Binding data at Gly/NMDA, AMPA, and high-affinity KA receptors showed that the presence of the free 3-hydroxy group is of paramount importance for a good affinity at all three investigated receptors, while introduction of some 6-heterocyclic moieties yielded AMPA-selective antagonists. The most significant result was the finding of the 6-(2-carboxybenzoylamino)-3-hydroxy-1*H*-quinazolin-2,4-dione **12**, which possesses good affinity for high-affinity and low-affinity KA receptors ($K_i = 0.62$ μM and 1.6 μM, respectively), as well as good selectivity. To rationalize the trend of affinities of the reported derivatives, an intensive molecular modeling study was carried out by docking compounds to models of the Gly/NMDA, AMPA, and KA receptors.

Introduction

Glutamate (Glu) is the primary excitatory neurotransmitter in both the central and the peripheral nervous systems where it plays a pivotal role in a host of physiological processes such as neuronal plasticity, learning, and memory. Glu exerts its effects by activation of metabotropic (mGluRs) and ionotropic receptors (iGluRs).[1,2] The iGluRs are 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 coagonist glycine binding site (Gly/NMDA).[3] To date, molecular cloning has identified six subunits for the NMDA receptor (NR1, NR2A−D, and N3A), four subunits for the AMPA receptor (GluR1−4), and five subunits (GluR5−7 and KA1−2) for the KA receptor.[1] KA1 and KA2 subunits showed high-affinity binding for [^{3}H]kainate,[4,5] while GluR5−7 are low-affinity subunits.[6,7] Excessive glutamatergic transmission is involved in the pathogenesis of several neurological disorders, including hypoxia/ischemia brain damage,[8] Parkinson's[9] and Alzheimer's[10,11]diseases, epilepsy,[1] and multiple sclerosis.[12−13] Accordingly, the blockade of iGluRs could be employed for the treatment of the aforementioned pathologies.[1,14−15] A large body of data also indicates that glutamatergic neurotransmission is involved in nociceptive reflexes at the spinal cord level.[16,17] In fact, the presence of NMDA, AMPA, and KA receptors in the dorsal horn of rat and human spinal cords has been well-documented,[18,19] and many studies have demonstrated the key role of the iGluRs for transmission of pain.[20−22] Indeed, it has been well-established that NMDA receptor antagonists have antinociceptive activity in different types of chronic pain, both in human and animal models, but their clinical use is limited due to unacceptable central side effects such as hallucination and ataxia.[23] Similarly, application of mixed AMPA/KA receptor antagonists as analgesics is generally not possible due to undesirable ataxia,[21,24] while KA receptor selective antagonists seem to have more specific antinociceptive effects, at least in rats,[24] thus making them more promising antinociceptive therapeutic agents.

In the last few decades, the research focused on iGluRs has acquired considerable insights on Gly/NMDA and AMPA receptors as a result of the availability of selective agonists and antagonists, which have permitted clarification of the physiological role of the two receptor types.[1,2,25−29] In contrast, the role of the KA receptor is much less understood because for a long time there was a lack of selective antagonists, and some KA receptor selective antagonists have only recently been reported.[29−33]

Recently, we have directed efforts toward the study of novel competitive and noncompetitive iGluR antagonists, with the aim of shedding light on the different structural requirements of the various receptor subtypes.[34−42] Recently, we published a paper where a preliminary study on 3-hydroxy-1*H*-quinazoline-2,4-dione derivatives was reported[40] (Chart 1). These derivatives possess all the structural requirements for Gly/NMDA and AMPA receptor recognition:[27,43] (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 2-carbonyl

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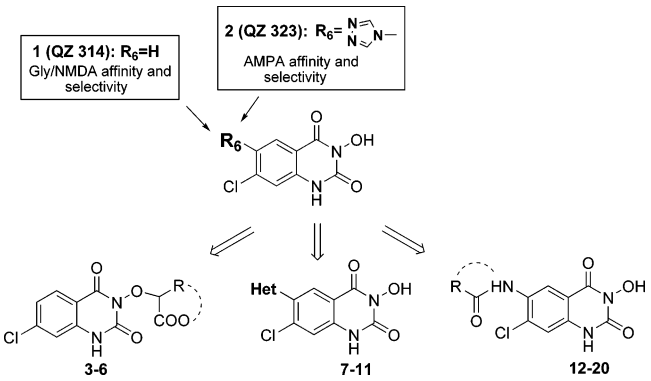
Chart 1. Previously and Currently Reported 7-Chloro-quinazoline-2,4-dione Derivatives



group and the 3-hydroxy substituent, which could form a hydrogen bond with a cationic hydrogen-bond donor receptor site. Among the previously reported compounds, the 7-chloro-3-hydroxy-1*H*-quinazoline-2,4-dione **1** (**QZ 314**, Chart 1) was found to be a Gly/NMDA receptor-selective antagonist. Introduction of the 6-(1,2,4-triazol-4-yl) residue on derivative **1** shifted the affinity and selectivity toward the AMPA receptor (compound **2**, i.e., **QZ 323**, Chart 1). In general, the 3-hydroxyquinazoline-2,4-dione derivatives showed lower affinities for the KA receptor than for the AMPA one.

Taking into account these findings, we decided to continue the study of this class of compounds to further explore the structure−affinity relationships (SAR) and gain greater insight about the differences among the structural requirements of the three receptor types, in particular those between AMPA and KA receptors. In fact, while known pharmacophore models of Gly/NMDA and AMPA receptor antagonists point out the different features of the two classes of ligands,[27,43] it has not yet been clarified how to shift the affinity from the AMPA to the KA receptor. Indeed, so far only a few KA- versus AMPA-selective antagonists have been reported,[31−33] despite being needed as tools for pharmacological characterization of the KA receptor. Thus, to address this issue, in this paper the study of the 7-chloro-quinazoline-2,4-dione derivatives **3**−**20** (Chart 1), designed as AMPA- and/or KA-receptor antagonists, is described. Compounds **3**−**6** are 3-*O*-modified analogues of derivative **1**. On the fused benzo ring compounds **7**−**11** bear some typical heterocyclic substituents of previously reported bicyclic and tricyclic selective AMPA- and KA-receptor antagonists.[27,28,32,36,38,39] In contrast, compounds **12**−**20** have some selected amide or ureide moieties at the 6-position, which we did not investigate in depth in our previously reported AMPA- and KA-receptor antagonists.

Chemistry

Compounds **3**−**20** were synthesized as described in Schemes 1−5. Scheme 1 depicts the synthesis of the 3-*O*-functionalized compounds **3**−**6**. Reaction of the 7-chloro-3-hydroxyquinazoline-2,4-dione **1**[40] with α-bromo-γ-butyrolactone in methyl ethyl ketone yielded to the 3-*O*-lactone derivative **3**, which was hydrolyzed to give the corresponding acid derivative **4**. The 3-*O*-(carbethoxymethyl)-substituted compound **5** was prepared by treating compound **1** with ethyl bromoacetate and triethylamine in ethanol. Alkaline hydrolysis of the ethyl ester **5** afforded the corresponding acid **6**.

Compounds **7**−**11** were obtained starting from the previously reported 6-amino-7-chloro-3-acetoxyquinazoline-2,4-dione **21**[40] (Scheme 2). Reaction of **21** with 2,5-diethoxytetrahydrofuran or 2,5-dimethoxytetrahydrofuran-3-carbaldehyde in glacial acetic acid at 90 °C gave, respectively, the 6-(pyrrol-1-yl)- derivative **22** and the 6-(3-formylpyrrol-1-yl)- derivative **23**. This latter was oxidized with potassium permanganate in a water/acetone mixture to the corresponding 6-(3-carboxypyrrol-1-yl)- derivative **24**. The 3-acetoxy derivatives **22**−**24** were hydrolyzed to the desired 3-hydroxy derivatives **7**−**9**. When the 6-amino compound **21** was reacted with 2,5-dimethoxy-2,5-dihydrofuran, in glacial acetic acid at 90 °C, the 6-(1,5-dihydropyrrol-2-oxo-1-yl) derivative **25** was obtained, which was deacetylated with a few drops of piperidine in boiling ethanol to give the 3-hydroxy- derivative **10**. The 6-(4-oxo-4*H*-pyridin-4-yl)- derivative **11** was prepared by heating the 6-amino compound **21** and the 4-oxo-4*H*-pyran-2,6-dicarboxylic acid (chelidonic acid) in dimethyl sulfoxide at 90 °C.

Compounds **12**−**14** were prepared as shown in Scheme 3. Reaction of the 6-amino derivative **21** with phthalic anhydride and succinic anhydride, in glacial acetic acid at 60 °C, gave, respectively, the 6-phthalimido- derivative **26** and the 6-succinamido- compound **27**. Alkaline hydrolysis of compounds **26** and **27**, followed by acidification with 6 N HCl, yielded the 3-hydroxy-6-phthalamido- derivative **12** and the 3-hydroxy-6-succinamido- derivative **13**. When the 6-succinamido- derivative **27** was reacted with sodium acetate in boiling acetic anhydride, the corresponding 6-succinimido- compound **28** was obtained, which was deacetylated to the corresponding 3-hydroxy derivative **14** with piperidine in refluxing ethanol.

The 6-aroylamino-3-hydroxyquinazoline-2,4-diones **15**−**19** were synthesized as depicted in Scheme 4. Reaction of the 6-amino-quinazoline-2,4-dione derivative **21**[40] with benzoyl chloride, 3-carbomethoxybenzoyl chloride,[44] or 4-carbomethoxybenzoyl chloride,[45] in anhydrous methylene chloride, gave rise to the corresponding 6-aroylamino-3-acetoxy-derivatives **29**, **30**, and **31**. Alkaline hydrolysis of **29**−**31** yielded compounds **15**−**17**, while treatment of compounds **30** and **31** with piperidine in boiling ethanol afforded compounds **18** and **19**.

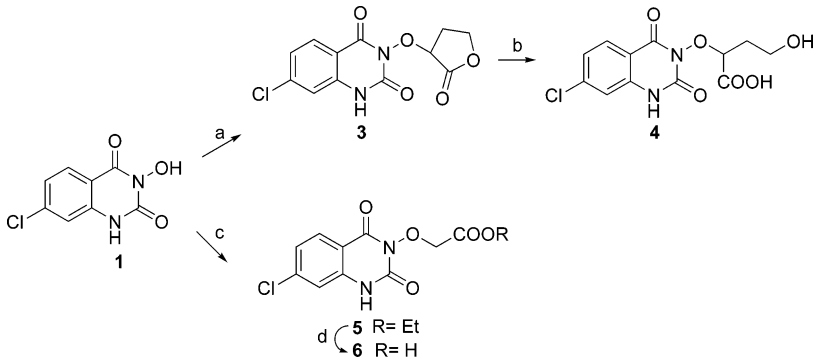
The 6-benzylureido-3-hydroxyquinazoline-2,4-dione **20** was prepared as shown in Scheme 5. By reacting the 6-amino derivative **21** with benzylisocyanate, the 3-acetoxy-6-benzylureido- compound **32** was obtained, which in alkaline medium yielded the desired 3-hydroxy derivative **20**.

Results and Discussion

The herein described 3-hydroxyquinazoline-2,4-diones **3**−**20** were designed taking the previously reported 7-chloro-3-hydroxyquinazoline-2,4-dione **1**[40] and the corresponding 6-(1,2,4-triazol-4-yl)- derivative **2**[40] as lead compounds. Compounds **3**−**20** were evaluated for their binding at AMPA, Gly/NMDA, and high-affinity KA receptors.

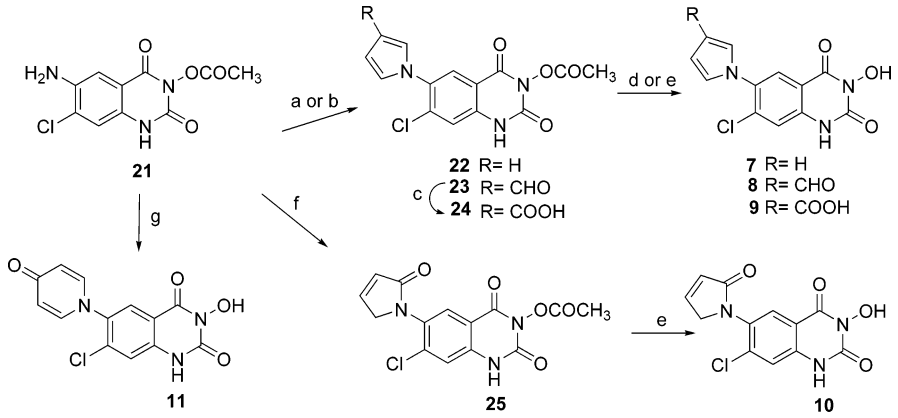
Compounds **3**−**6** are 3-*O*-modified analogues of derivative **1** (Table 1). This set of compounds was synthesized to evaluate the effects elicited by functionalization of the 3-hydroxy group with carboxy-containing alkyl chains. The idea of introducing such substituents on the 3-hydroxy group sprang from the binding data of compound **33 (SPD 502)**[46,47] (Figure 1, Table 1), which belongs to the class of the 3-isatineoximes, described as AMPA/KA receptor antagonists.[48] Differently from the other 3-isatinoxime analogues, which bear the unsubstituted oxime group, derivative **33** presents an attached carboxylic side chain on the oxime function. This modification, made to improve water solubility of the molecule, afforded high AMPA affinity and selectivity.[46] Because the 3-isatinoxime moiety displays some similarities with the 3-hydroxy-quinazoline-2,4-dione scaffold, we presumed a similar binding mode for these two

Scheme 1



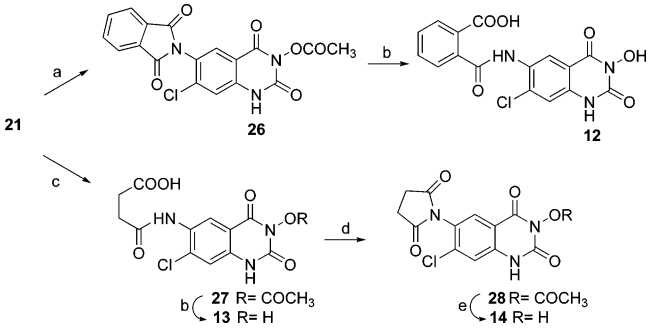
(a) (i) α-Bromo-γ-butyrolactone, K_2CO_3, ethyl methyl ketone, 90 °C; (ii) 6 N HCl; (b) (i) 1% NaOH, rt; (ii) 6 N HCl; (c) ethyl bromoacetate, NEt_3, EtOH, rt; (d) (i) 2.5% NaOH, rt; (ii) 6 N HCl.

Scheme 2



(a) 2,5-Diethoxytetrahydrofuran, glacial AcOH, 90 °C; (b) 2,5-dimethoxytetrahydrofuran-3-carbaldehyde, glacial AcOH, 90 °C; (c) (i) $KMnO_4$, H_2O/acetone (1:1), 0 °C; (ii) 38% sodium hydrogen sulfite; (iii) 6 N HCl; (d) (i) 2.5% NaOH, rt; (ii) 6 N HCl; (e) piperidine, EtOH, reflux; (f) 2,5-dimethoxy-2,5-dihydrofuran, NaOAc, glacial AcOH, 90 °C; (g) 4-oxo-4*H*-pyran-2,6-dicarboxylic acid (chelidonic acid), DMSO, 90 °C.

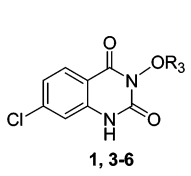
Scheme 3



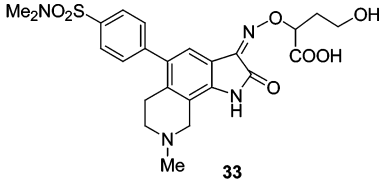
(a) (i) Phthalic anhydride, NaOAc, glacial AcOH, 60 °C; (b) (i) 1% NaOH, rt; (ii) 6 N HCl; (c) (i) succinic anhydride, NaOAc, glacial AcOH, 60 °C; (ii) 6 N HCl; (d) NaOAc, Ac_2O, reflux; (e) piperidine, EtOH, reflux.

classes of compounds. Thus, we decided to first introduce the same 2-(4-hydroxybutanoic) acid chain of derivative **33** on the 3-hydroxy group of the quinazoline-2,4-dione **1** (derivative **4**). In contrast with the isatinoxime derivative **33**, compound **4** completely lacked affinity at all three investigated receptors. The same applies to the corresponding 3-*O*-lactone derivative **3**, a conformationally constrained analogue of derivative **4**. When the acetic acid side chain was positioned on the 3-hydroxy group of derivative **1** (compound **6**) some Gly/NMDA and AMPA affinities were restored, while esterification of the acetic group of compound **6** resulted in a complete loss of affinity at all three receptors (compound **5**). In summary, the scarce/null affinities of the 3-*O*-substituted derivatives **3**−**6** highlight that in this class of compounds the free 3-hydroxy group plays a pivotal role for anchoring to AMPA, Gly/NMDA, and KA receptors.

Table 1. Binding Affinity at AMPA, Gly/NMDA, and High-affinity KA (KA_{h-a}) Receptors



1, 3-6

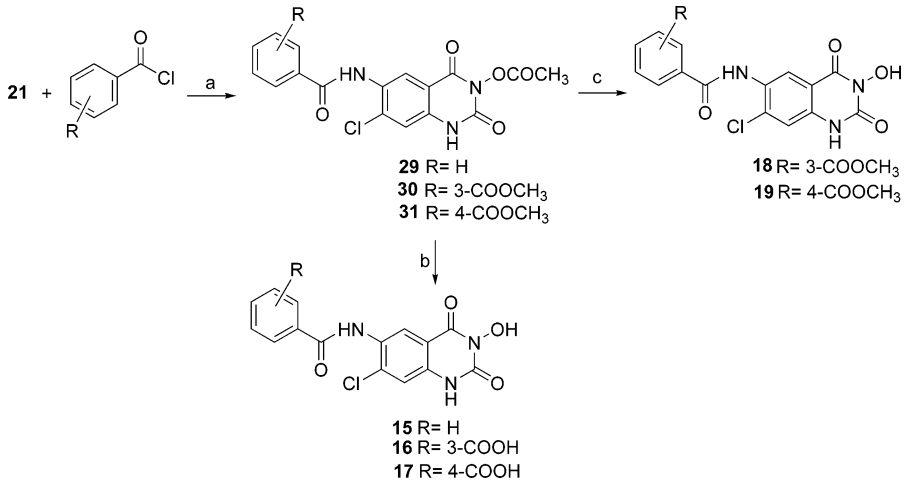


33

	R_3	K_i (μM)[a] or I%[b] [^{3}H]AMPA	[^{3}H]glycine	IC_{50}(μM)[c] [^{3}H]KA_{h-a}
1[d]	H	11.6 ± 1.9	0.24 ±0.02	140 ± 15
3		26%	37%	11%
4		35%	40%	10%
5	CH_2COOEt	9%	0%	5%
6	CH_2COOH	99 ± 7	90 ± 7	5%
33[e]		0.043 ± 0.007	>30	81 ± 12

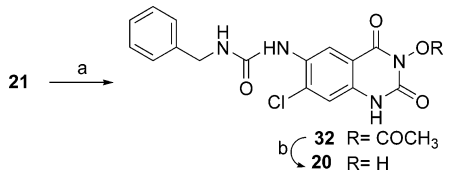
[a] K_i values are means ± SEM of three or four separate determinations in triplicate. [b] Percentage of inhibition (I%) of specific binding at 100 μM concentration. [c] IC_{50} values are means ± SEM of three or four separate determinations in triplicate. [d] Reference 40. [e] Reference 46.

Scheme 4



(a) Anhydrous CH_2Cl_2, pyridine, 0 °C; (b) (i) 2% NaOH, rt; (ii) 6 N HCl; (c) piperidine, EtOH, reflux.

Scheme 5



(a) Benzylisocyanate, anhydrous THF, reflux; (b) (i) 1% NaOH, rt; (ii) 6 N HCl.

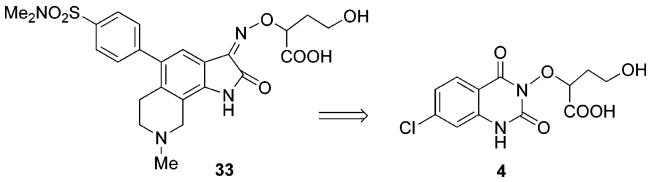
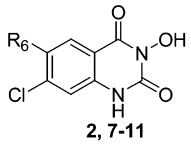


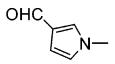
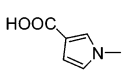
Figure 1. 3-Isatinoxime derivative **33**-based structure of compound **4**.

The set of derivatives **7**−**11** was designed taking as lead compound the previously reported AMPA selective antagonist **2**[40] (Table 2). In fact, new compounds formally resulted from the replacement of the 6-(1,2,4-triazol-4-yl) group of the lead with other heterocyclic rings, some of which were profitable in well-known classes of AMPA-receptor antagonists.[27,28,38,41] The binding data of compounds **7**−**11**, reported in Table 2, show that, as expected, the presence of a suitable 6-heterocyclic residue shifted the affinity toward the AMPA receptor, the only exception being the pyrrol-1-yl group (compound **7**), which afforded higher affinity for the Gly/NMDA site than for the AMPA receptor. The 6-(4-oxo-pyridin-1-yl)-derivative **11** displayed the highest AMPA receptor affinity, comparable to that of the lead compound **2**. KA receptor affinities of derivatives **7**−**11** are similar (**7**, **8**, and **10**) or lower (**9** and **11**) than the AMPA ones.

Compound **11**, which showed the highest AMPA receptor affinity, was tested to evaluate its antagonistic activity by assessing its ability to inhibit depolarization induced by 5 μM AMPA and NMDA in cortical wedge preparations.[38] The results obtained from these functional antagonism studies are reported in Table 3, where the inhibitory potencies of **2** and NBQX (2,3-dihydroxy-6-nitro-7-sulfamoylbenzo[*f*]quinoxaline) have also been included. Compound **11** inhibits AMPA and NMDA responses in a reversible manner. Its inhibitory activities are similar to those of the lead compound **2** and are in agreement with its binding affinities. In fact, compound **11** shows greater potency in inhibiting AMPA-evoked response than NMDA-induced depolarization.

Table 2. Binding Affinity at AMPA, Gly/NMDA, and High-affinity KA (KA_{h-a}) Receptors



		K_i (μM)[a] or I%[b]		IC_{50}(μM)[c]
	R_6	[^{3}H]AMPA	[^{3}H]glycine	[^{3}H]KA_{h-a}
2[d]		0.25 ± 0.04	47%	7.0 ± 0.5
7		16.6 ± 0.9	1.0 ± 0.09	8.2 ± 0.8
8		4.2 ± 0.1	22.7 ± 2.7	6.2 ± 0.4
9		1.3 ± 0.2	44%	13 ± 0.6
10		6.2 ± 0.1	42 ± 11	10 ± 0.8
11		0.5 ± 0.09	25 ± 3	4.1 ± 0.5

[a] K_i values are means ± SEM 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 means ± SEM of three or four separate determinations in triplicate. [d] Reference 40.

Table 3. Functional Antagonism of Derivative **11** at NMDA and AMPA Sites

	IC_{50}[a] (μM)	
	AMPA	NMDA
11	3.5 ± 0.4	>80
2[b]	4.6 ± 0.5	84 ± 10
NBQX	0.20 ± 0.02	(*)[c]

[a] Concentration necessary for 50% inhibition (IC_{50}) of depolarization induced by *S*-AMPA or NMDA in mouse cortical wedge preparation. IC_{50} values are means ± SEM of three separate determinations. [b] Reference 40. [c] At 10 μM concentration, the inhibition was not significant.

To interpret the AMPA binding affinities of derivatives **7**−**11**, we performed a molecular modeling study by docking these compounds, and the leads **1** and **2**, in the domain-open state of the GluR2 ligand-binding core (GluR2−S1S2J/(*S*)-ATPO crystal structure, pdb entry **1n0t**),[49] ((*S*)-ATPO, namely, (*S*)-2-amino-

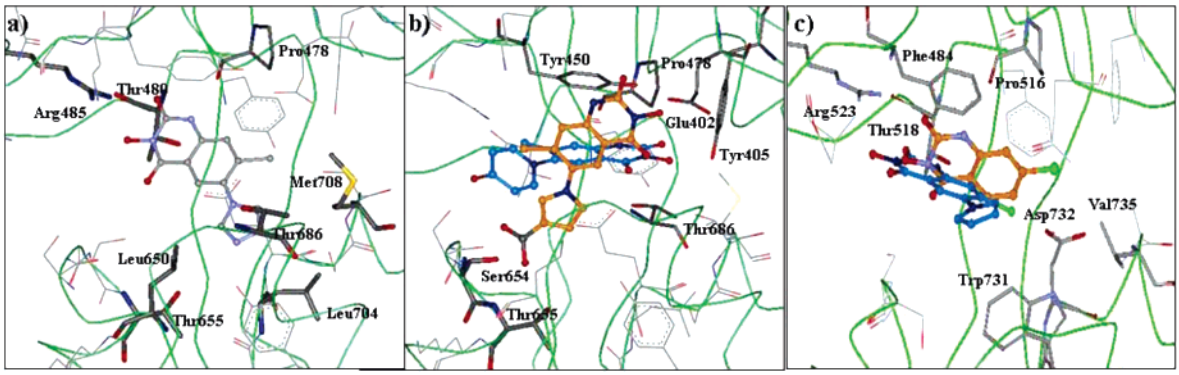


Figure 2. Compound **2** (panel a) and compounds **9** and **11** (orange and blue, respectively; panel b) docked into the X-ray GluR2−S1S2J/(*S*)-ATPO crystal structure (1n0t). Best docking conformation of compounds **1** (orange) and **7** (blue) in the Gly/NMDA receptor binding site (panel c). The ligand is shown in ball-and-stick representation. Side chains of receptor residues involved in the interactions are in stick representation. Standard atomic colors are used (carbon = gray, oxygen = red, nitrogen = blue, sulfur = yellow, chlorine = green).

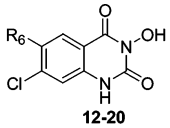
3-[5-*tert*-butyl-3-(phosphonomethoxy)-4-isoxazolyl] propionic acid). The antagonist binding site of glutamate ionotropic receptors (iGluRs) is located in a clam shell-like ligand binding region composed of two domains, domain 1 and domain 2. The interactions of compounds **1**, **2**, and **7** with the AMPA receptor binding pocket involve the highly conserved residue triad among iGluRs: Arg485, Thr480, and Pro478 (numbering as in ref 49; Figure 2, panel a; for clarity only compound **2** is shown). In particular, the NH group of these derivatives engages a hydrogen bond with Pro478 (backbone CO), the 2-carbonyl residue interacts with Arg485 and Thr480 (guanidine side chain and backbone NH, respectively), and the 3-hydroxy group interacts with the guanidine residue of Arg485. Moreover, the aromatic portion of the quinazoline scaffold is involved in a $\pi-\pi$ stacking interaction with the aromatic moiety of Tyr450, located at the top of the binding pocket. In addition, the 6-heterocyclic rings of both **7** and **2** occupy the binding cleft formed by Leu650, Leu704, Thr655, Thr686, and Met708. Interestingly, the electron deficiency of the 6-(1,2,4-triazol-1-yl) moiety of derivative **2** determines an additional interaction[50] with the hydroxy group of the Thr686 side chain. This interaction is likely to reinforce the anchoring of this compound to the above-mentioned pocket and, at least partly, might account for the higher AMPA receptor affinity of **2** with respect to that of the 6-pyrrol derivative **7**.

Very unexpectedly, the anchoring of derivatives **9** and **11** to the AMPA receptor binding site (Figure 2, panel b) was different with respect to that of derivatives **7** and **2**. Moreover, **9** and **11** also differ between them for their binding mode. In compound **11**, the NH group acts as a H-bond donor in favor of Pro478 (backbone CO), and the 2-carbonyl oxygen forms a hydrogen bond with the hydroxy group of Tyr405, though the distance is somewhat greater (3.2 Å) than a typical H-bond interaction. The 3-hydroxy substituent and the 4-carbonyl group interact, respectively, with Glu402 and Thr686, thus preventing interaction between these interdomain residues, an interaction that is crucial to stabilize the closed agonist state conformation of GluR2−S1S2J. The position and distance (about 3.7 Å) of the aromatic quinazoline ring of **11** are optimal for a $\pi-\pi$ stacking interaction with the aromatic Tyr450 residue. In regard to compound **9**, the NH and 2-carbonyl groups seem to not make significant interactions with the recognition site, while the 3-hydroxy and 4-carbonyl groups form hydrogen bonds with Glu402 and Thr686, respectively, thus avoiding the domain closure and holding the antagonist-binding core in its open-cleft conformation. The 6-substituent of **9** interacts, through the carboxy group, with both Ser654 and Thr655.

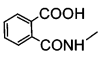
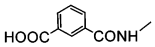
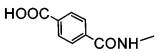
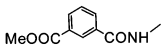
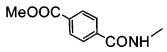
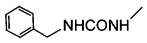
To interpret the trend of Gly/NMDA receptor affinity of compounds **7**−**11**, we docked these derivatives, and the lead compounds **1** and **2**, to a recently described model of the Gly/NMDA receptor.[42] The peculiar interactions between these compounds and the Gly/NMDA receptor binding pocket are very similar to the interactions found for the GluR2 receptor on account of the fact that the highly conserved triad is involved in the most significant hydrogen bonds. Briefly, in the Gly/NMDA receptor, the residues of the triad are Arg523, Thr518, and Pro516 (numbering as in ref 51). Considering compound **1** the most active ligand at the Gly/NMDA receptor among this series, the NH group acts as a proton donor in favor of Pro516 (backbone CO), while the 2-carbonyl group accepts a hydrogen bond from Thr518 (backbone NH; Figure 2, panel c). Both the 2-carbonyl and the 3-hydroxy groups of this compound interact with Arg523 (guanidine residue). Moreover, at the top of the binding pocket, the aromatic ring of Phe484 makes $\pi-\pi$ stacking interaction with the aromatic quinazoline scaffold. The 6-pyrrole-substituted derivative **7** shows a quite similar binding mode to that of compound **1** (Figure 2, panel c). Indeed, **7** can still interact with Thr518 and Arg523 residues through the 2-carbonyl and 3-hydroxy groups. Moreover, the 6-pyrrole ring is settled in a cavity formed by nonconserved residues such as Val735, Asp732, and Trp731.[42] This subsite is less roomy and more hydrophobic than the corresponding pocket in the GluR2−S1S2J receptor, especially due to the steric hindrance of the Trp731 indole moiety. For this reason, only the pyrrole ring seems to be small enough to find space in the opening between lobe S1 and lobe S2, very close to Trp731, making a hydrophobic interaction with the aromatic side chain of this residue and avoiding the closure of the ligand binding cleft. In contrast, the 6-substituents of compounds **2** and **8**−**11** cannot fit in this subsite, forcing compounds to find an alternative and less-effective conformation in the binding site.

To evaluate the effect of nonheterocyclic substituents at the 6-position, we synthesized the set of derivatives **12**−**20** that bear different 6-amide or 6-ureide moieties. The binding results of derivatives **12**−**20** are reported in Table 4, where the binding affinities of the willardiine analogue **UBP 302**[52,53] (compound **35**, Figure 3) are also reported. The binding data show that the 6-amide and 6-ureide substituents shifted affinities toward the AMPA and KA receptors. As the most important result, we have obtained a potent and selective KA receptor antagonist: the 6-(2-carboxybenzoylamino)-3-hydroxy-1*H*-quinazolin-2,4-dione **12 (QZ 443)**. This compound possesses good affinity and selectivity for the KA high-affinity receptor. Compound **12** was designed as an analogue of derivative **9** (Figure 3). In fact,

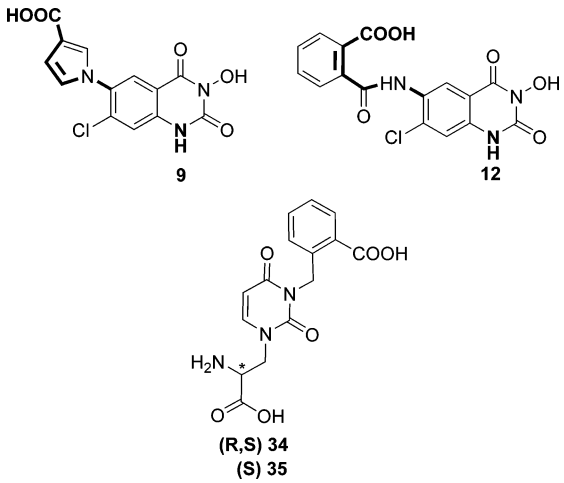
Table 4. Binding Affinity at AMPA, Gly/NMDA, High-Affinity KA (KA_{h-a}), and Low-Affinity KA (KA_{l-a}) Receptors



12-20

		K_i (μM)[a] or I%[b]		IC_{50}(μM)[c] or I%[b]	
	R_6	[^{3}H]AMPA	[^{3}H]glycine	[^{3}H]KA_{h-a}	[^{3}H]KA_{l-a}
12		16.5 ± 1.2	40%	0.62 ± 0.02	1.6 ± 0.5
13		8.4 ± 1.0	8%	5.4 ± 0.2	
14		36 ± 4	88 ± 10	44%	
15		45 ± 17	33%	52 ± 5	
16		60 ± 10	32%	100 ± 9	0%
17		29%	40%	12.9 ± 0.9	0%
18		35%	32%	20%	
19		43%	10%	28%	
20		33%	27%	32%	
(S) 35		117 ± 15	20%	46 ± 5	15 ± 4

[a] K_i values are means ± SEM 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 means ± SEM of three or four separate determinations in triplicate.



9 **12** **(R,S) 34** **(S) 35**

Figure 3. Compounds **9**, **12**, and (*R*,*S*)-3-(2-carboxybenzyl)-willardiine **34** and its (*S*)-enantiomer **35**.

compounds **12** and **9** share a carboxy group in a position that is similarly spaced from the 3-hydroxy-quinazoline-2,4-dione scaffold. Differently from **9**, the carboxy function of derivative **12** was inserted on a benzo ring that was moved away from the 3-hydroxyquinazolin-2,4-dione nucleus, by means of the carbamoyl spacer, with the purpose of enhancing the volume of the molecule in the neighborhood of the 6-position. Interestingly, this modification shifted affinity and selectivity from the AMPA (compound **7**) to the KA receptor (compound **12**). Compound **12** bears the *ortho*-carboxyphenyl moiety, similarly to the willardiine analogue (*R*,*S*)-3-(2-carboxybenzyl)-willardiine **34** (**UBP 296**, Figure 3), which was recently reported in the literature[52,53] as a selective antagonist of the KA receptor. Compound **34** was found to be a potent and selective antagonist of the native GluR5-containing KA receptor in the spinal cord, with activity residing in the *S*-enantiomer **35**. Because no affinity data at native KA receptors were reported for either **35** or its racemate **34**,[52,53] we tested the commercially available **35** in our experiments to evaluate its binding at the high-affinity native KA receptor, which is described to be represented by KA1 and KA2 subunits.[4,5,6,54] Compound **35** was also tested at AMPA and Gly/NMDA receptors. The binding results, reported in Table 4, showed that **35** possesses, respectively, low and null affinity for AMPA and Gly/NMDA receptors. This derivative also has low affinity for the high-affinity KA receptor, about 75-fold lower than that of compound **12**. Compound **12** and the willardiine derivative **35** were also tested at the low-affinity KA binding site,[54] which consists of a combination of GluR5−7 subunits.[6,7] As the binding results indicate (Table 4), derivatives **12** and **35** show, respectively, a halved and 3-fold higher affinity at the low-affinity KA receptor with respect to the high-affinity one. Most importantly, compound **12** possesses higher affinity than derivative **35**, even at the low-affinity site. It also has to be highlighted that derivative **12**, compared to **35**, possesses a higher selectivity toward both high- and low-affinity KA sites with respect to the AMPA receptor.

The presence and position of the distal acidic carboxy group play a critical role for KA receptor affinity and selectivity of derivative **12**. In fact, removal of this substituent (compound **15**) or its movement from the ortho to the meta (compound **16**) or to the para position (compound **17**) significantly decreased the binding at the high-affinity KA receptor. However, it has to be noted that derivative **17** still maintains higher affinity and selectivity for this receptor with respect to those of the willardiine derivative **35**. Both compounds **16** and **17** are, instead, totally unable to bind to the low-affinity KA site.

The replacement of the lipophilic benzene linker, between the 6-carbamoyl spacer and the acidic distal function of **12**, with the more flexible ethylene chain afforded a 10-fold reduced KA receptor affinity (compound **13**), probably due to the free rotation of the ethylene chain. Indeed, the benzene ring of derivative **12** might constrain the carboxy group in the right position for a good hydrogen-bonding interaction. Cyclization of the 6-succininamido chain of **13** to afford derivative **14** drastically reduced affinity toward the KA receptor and, to a lesser extent, toward the AMPA one. Similarly, a complete loss of KA affinity ensued from esterification of the carboxy group of compounds **16** and **17** (see derivatives **18** and **19**). Also derivative **20**, which bears the long 3-benzylureido side chain at the 6-position, completely lacks AMPA and KA receptor affinities.

Due to the interesting binding affinity and selectivity of derivative **12** at KA receptors, both high- and low-affinity ones, we decided to evaluate its antinociceptive effect; it is well-known that KA receptor antagonists are effective as analgesics in various animal models of pain.[21,24] Thus, compound **12** was tested in the acetic acid-induced writhing assay and the results are reported in Table 5. The effect of diclofenac (2-[(2,6-dichlorophenyl)amino]benzeneacetic acid) is also reported for comparison. Compound **12** showed antinociceptive properties because it increased the pain threshold in a dose-dependent manner starting from the dose of 1.0 mg kg^{-1} po. The dose of 0.1 mg kg^{-1} was devoid of any effect. Compound **12**, at doses

Table 5. Effect of Compound **12** In Comparison with Diclofenac in the Mouse Abdominal Constriction Test

treatment per os[a]	no. mice	dose mg kg^{-1}	no. writhes
CMC[b]	12		35.1 ± 3.7
12	8	0.1	34.7 ± 2.9
12	11	1.0	26.1 ± 3.3[c]
12	12	10	24.5 ± 3.1[c]
diclofenac	14	5.0	18.2 ± 3.6[c]

[a] Compound **12** was administered 30 min before test. [b] Carboxymethylcellulose. [c] $P < 0.01$ versus CMC-treated mice.

Table 6. Effect of Compound **12** on Mice Rota-Rod Test

			after treatment[a]		
treatment per os	dose mg kg^{-1}	before treatment[a]	30 min	45 min	60 min
CMC[b]		5.6 ± 0.4	4.0 ± 0.3	3.7 ± 0.2	2.0 ± 0.2
12	20	5.1 ± 0.3	3.3 ± 0.4	2.6 ± 0.2	1.7 ± 0.3
12	50	4.8 ± 0.3	4.8 ± 0.2	3.7 ± 0.3	3.2 ± 0.3

[a] Number of falls from the rod in 30 s. Each value represents the mean of eight mice. [b] Carboxymethylcellulose.

20−50 times higher than those effective in increasing the pain threshold, was unable to modify mouse motor coordination, evaluated by means of the rota-rod test (Table 6). These results not only rule out the possibility that the antinociceptive effect observed was related to an altered viability of treated animals, but also evidence a good tolerability of compound **12** in laboratory animals.

In an attempt to depict the binding mode of compound **12** at the low-affinity KA receptor, the building of a homology model of the antagonized state of the receptor was undertaken. In fact, only the X-ray structures for the closed agonized state of GluR5 and GluR6 binding domains were available.[55,56] Thus, taking advantage of the almost identical secondary structures and the high similarity in amino acidic sequence (50%) of the AMPA receptor versus the KA receptor, a GluR5 homology model was derived from the open state of the GluR2 ligand-binding core. Passing from the GluR2 ligand binding pocket to the GluR5 one there is the exchange of five residues, all belonging to domain 2, namely, Leu650 to Val670, Thr686 to Ser706, Tyr702 to Leu720, Leu704 to Met722, and Met708 to Ser726. These changes make the volume of the binding cavity of GluR5 40% larger than the volume of GluR2, increasing the number of trapped water molecules and making the chemical features of some subpockets in the two receptor types different.[56]

The results of docking experiments on compound **12** in the homology-built model of GluR5 (hmGluR5; Figure 4) point out the interaction of the 6-carbamoyl CO group with the guanidine residue of Arg508. The *ortho*-carboxy moiety accommodates in the protein region favorable for a negatively charged group, thus establishing a direct hydrogen bond with Ser674. The substitution of the Met708 and Thr686 residues in GluR2 by Ser726 and Ser706 in GluR5 permits a deeper insertion of the quinazoline ring of **12** into the GluR5 binding pocket than in the GluR2 one (not shown in figures), thus allowing a direct interaction of the 3-OH group with the side chain of Glu426. The quinazoline ring of **12** lies about 3.7 Å below and parallel to the aromatic moiety of Tyr474, thus forming a $\pi-\pi$ stacking interaction. Moreover, it is likely that the formation of an intramolecular hydrogen bond between the carboxy group and the carbamoyl NH further stabilizes the bound conformation of the compound.

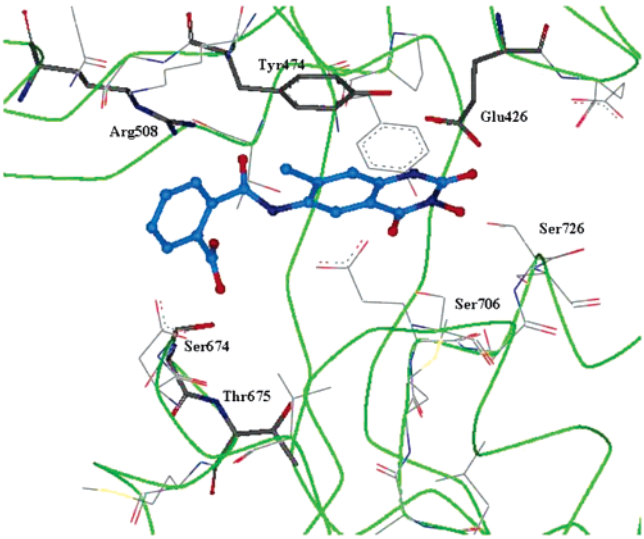


Figure 4. Compound **12** docked into a homology model of GluR5/ATPO built from the X-ray structure of the GluR2/DNQX and GluR2/ATPO complexes. The ligand and receptor residues are represented with the same coloring scheme for the atoms as in Figure 2.

Conclusion

Synthesis of the herein reported compounds has allowed us to further explore the SAR in this class of derivatives, and it has also afforded some selective AMPA receptor antagonists characterized by good affinities and selectivities. However, the most significant result of this work is the finding of a new KA receptor antagonist (compound **12**) that possesses good affinity for both high- and low-affinity KA receptors and also good selectivity not only toward the Gly/NMDA receptor, but also toward the AMPA one. The importance of this result resides in the fact that, currently, only a few examples of KA-receptor-selective antagonists are known. It should also be highlighted that in our binding experiments at the native KA receptor, derivative **12** shows higher affinity than the willardiine analogue **35** that was recently reported as a potent and selective antagonist at the native GluR5-containing KA receptor in rat spinal cord. Modeling studies have provided valuable evidence of the putative binding modes of the synthesized antagonists at all three investigated receptors, pointing out different sets of interactions that involve different amino acidic residues and contribute to the affinity of the compounds. These studies prove themselves as useful tools for guiding the design and synthesis of new AMPA and KA receptor antagonists.

Experimental Section

Chemistry. Silica gel plates (Merck F254) were used for analytical chromatography. 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, 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. The following abbreviations are used: s = singlet, d = doublet, dd = double doublet, t = triplet, m = multiplet, br = broad, ar = aromatic protons, and al = aliphatic protons.

(±)- 7-Chloro-3-[(2-oxo-tetrahydrofuran-3-yl)oxy]-1*H*-quinazoline-2,4-dione (3). K_2CO_3 (1.88 mmol) and (±)-α-bromo-γ-butyrolactone (1.1 mL) were added to a hot (90 °C) suspension of 3-hydroxy-7-chloro-1*H*-quinazoline-2,4-dione **1**[40] (1.88 mmol) in ethyl methyl ketone (30 mL). The suspension was refluxed for 48

h. After cooling at room temperature, the suspension was diluted with water and acidified with 6 N HCl. The mixture was extracted with ethyl acetate (20 mL × 3), and the collected organic layers were anhydrified (Na_2SO_4) and evaporated at reduced pressure to give an oil. Upon treatment with a small amount of ethanol, a solid precipitated that was collected and recrystallized. Yield: 50%; mp 244−245 °C (CH_3CN). ^{1}H NMR: 2.38−2.46 (m, 1H, al), 2.59−2.68 (m, 1H, al), 4.32−4.36 (m, 1H, al), 4.45−4.49 (m, 1H, al), 5.07 (dd, 1H, al, J = 7.3, 5.0 Hz), 7.21 (d, 1H, H-8, J = 1.9 Hz), 7.30 (dd, 1H, H-6, J = 8.5, 1.9 Hz), 7.95 (d, 1H, H-5, J = 8.5 Hz), 11.85 (s, 1H, NH). IR 1694, 1733, 1773, 3206. Anal. ($C_{12}H_9ClN_2O_5$) C, H, N.

(±)-2-[(7-Chloro-2,4-dioxo-2,4-dihydro-1*H*-quinazolin-3-yl)-oxy]-4-hydroxybutanoic Acid (4). Derivative **3** (1.7 mmol) was dissolved in 1% aqueous NaOH (20 mL), and the solution stirred at room temperature for 30 min. After acidification with 6 N HCl, the mixture was extracted with ethyl acetate (20 mL × 3). The collected organic layers were anhydrified (Na_2SO_4) and evaporated at reduced pressure to yield a solid that was treated with cyclohexane (2−3 mL) and a few drops of ethyl acetate and collected by filtration. The crude product cannot be recrystallized since it changes into derivative **3**. Thus, derivative **4** was purified by acid−base exchange as follows: it was dissolved in 1% NaOH (about 20 mL), and the solution was extracted with ethyl acetate (about 20 mL). The aqueous phase was acidified to pH 1 with 6 N HCl and extracted with ethyl acetate (10 mL × 3). The organic layers were anhydrified (Na_2SO_4), and the solvent was evaporated at reduced pressure. The residue was treated with cyclohexane/ethyl acetate to yield a solid that was collected and dried. Yield: 74%; mp 241−245 °C, after decomposition at about 150 °C. ^{1}H NMR: 1.91−2.09 (m, 2H, al), 3.50−3.56 (m, 1H, al), 3.62−3.68 (m, 1H, al), 4.55 (br s, 1H, OH), 4.61 (t, 1H, al, J = 6.4 Hz), 7.20 (d, 1H, H-8, J = 1.8 Hz), 7.29 (dd, 1H, H-6, J = 8.5, 1.8 Hz), 7.94 (d, 1H, H-5, J = 8.5 Hz), 11.77 (s, 1H, NH), 12.89 (br s, 1H, COOH). IR: 1681, 1732, 1758, 3070−3420. Anal. Calcd for $C_{12}H_{11}ClN_2O_6$: C, 45.80; H, 3.53; N, 8.90. Found: C, 46.40; H, 3.90; N, 8.40.

Ethyl (7-Chloro-2,4-dioxo-2,4-dihydro-1*H*-quinazolin-3-yl)-oxyacetate (5). Ethyl bromoacetate (4.7 mmol) and triethylamine (4.7 mmol) were added to a suspension of compound **1**[40] (4.7 mmol) in ethanol (60 mL). The mixture was stirred at room temperature for 5 h, then the solid was collected and recrystallized. Yield: 57%; mp 183−185 °C (EtOH). ^{1}H NMR: 1.23 (t, 3H, CH_3, J = 7.1 Hz), 4.19 (q, 2H, CH_2, J = 7.1 Hz), 4.72 (s, 2H, CH_2), 7.20 (d, 1H, H-8, J = 1.9 Hz), 7.29 (dd, 1H, H-6, J = 8.5, 1.9 Hz), 7.94 (d, 1H, H-5, J = 8.5 Hz), 11.78 (s, 1H, NH). IR: 1674, 1732, 1760, 3192. Anal. ($C_{12}H_{11}ClN_2O_5$) C, H, N.

(7-Chloro-2,4-dioxo-2,4-dihydro-1*H*-quinazolin-3-yl)oxyacetic Acid (6). A suspension of the ester **5** (1.0 mmol) in 2.5% aqueous NaOH (20 mL) was stirred at room temperature for 1 h. The cooled (0 °C) solution was acidified to pH 1 with 6 N HCl, and the resulting solid was collected, abundantly washed with water, and recrystallized. Yield: 96%; mp > 300 °C (EtOH). ^{1}H NMR: 4.63 (s, 2H, CH_2), 7.21 (d, 1H, H-8, J = 1.9 Hz), 7.28 (dd, 1H, H-6, J = 8.5, 1.9 Hz), 7.95 (d, 1H, H-5, J = 8.5 Hz), 11.79 (br s, 1H, NH). IR: 1685, 1720, 1775, 3050−3290. Anal. ($C_{10}H_7ClN_2O_5$) C, H, N.

3-Acetoxy-7-chloro-6-(pyrrol-1-yl)-1*H*-quinazoline-2,4-dione (22). A solution of 2,5-diethoxytetrahydrofuran (2.2 mmol) in glacial acetic acid (3 mL) was dropwise added to a hot (90 °C) suspension of the 6-amino derivative **21**[40] (0.74 mmol) in glacial acetic acid (8 mL). The solution was heated at 90° C for 20 min, then most of the solvent was distilled off at reduced pressure. Dilution with water of the residue solution yielded a solid that was collected and recrystallized. Yield: 87%; mp 248−250 °C (EtOH). ^{1}H NMR: 2.39 (s, 3H, CH_3), 6.24 (s, 2H, pyrrole protons), 7.01 (s, 2H, pyrrole protons), 7.41 (s, 1H, ar), 7.83 (s, 1H, ar), 12.15 (br s, 1H, NH). Anal. ($C_{14}H_{10}ClN_3O_4$) C, H, N.

7-Chloro-3-hydroxy-6-(pyrrol-1-yl)-1*H*-quinazoline-2,4-dione (7). A solution of the 3-acetoxy derivative **22** (0.8 mmol) in aqueous 2.5% NaOH (20 mL) was stirred at room temperature for 1 h and then acidified to pH 1 with 6 N HCl. The resulting solid was collected, washed with water, and recrystallized. Yield: 85%; mp 242−243 °C (EtOH). ^{1}H NMR: 6.23 (s, 2H, pyrrole protons), 6.98 (s, 2H, pyrrole protons), 7.38 (s, 1H, ar), 7.79 (s, 1H, ar), 10.85 (br s, 1H, OH), 11.80 (s, 1H, NH). IR: 1690, 1750, 3100−3600. Anal. ($C_{12}H_8ClN_3O_3$) C, H, N.

3-Acetoxy-7-chloro-6-(3-formylpyrrol-1-yl)-1*H*-quinazoline-2,4-dione (23). A solution of 2,5-dimethoxytetrahydrofuran-3-carbaldehyde (2.7 mmol) in glacial acetic acid (7 mL) was dropwise added to a hot (90 °C) suspension of the 6-amino derivative **21**[40] (1.8 mmol) in glacial acetic acid (8 mL). After the addition was completed, the mixture was stirred at room temperature for 30 min. Evaporation of the solvent at reduced pressure yielded a solid that was suspended in water (10−20 mL), collected, and recrystallized. Yield: 70%; mp 233−234 °C (EtOH). ^{1}H NMR: 2.39 (s, 3H, CH_3), 6.65 (s, 1H, pyrrole proton), 7.17 (s, 1H, pyrrole proton), 7.44 (s, 1H, ar), 7.93 (s, 1H, pyrrole proton), 8.00 (s, 1H, ar), 9.76 (s, 1H, CHO), 12.30 (br s, 1H, NH). IR: 1670, 1710, 1740, 1820, 3120, 3250. Anal. ($C_{15}H_{10}ClN_3O_5$) C, H, N.

7-Chloro-3-hydroxy-6-(3-formylpyrrol-1-yl)-1*H*-quinazoline-2,4-dione (8). The title compound was obtained from **23** (0.8 mmol) following the experimental procedure described above to prepare **7** from **22**. Yield: 80%; mp > 300 °C (EtOH). ^{1}H NMR: 6.65 (s, 1H, pyrrole proton), 7.17 (s, 1H, pyrrole proton), 7.39 (s, 1H, ar), 7.93 (s, 1H, pyrrole proton), 7.96 (s, 1H, ar), 9.76 (s, 1H, CHO), 10.80 (br s, 1H, OH), 11.85 (br s, 1H, NH). IR: 1690, 1740, 3100−3600. Anal. ($C_{13}H_8ClN_3O_4$) C, H, N.

3-Acetoxy-7-chloro-6-(3-carboxypyrrol-1-yl)-1*H*-quinazoline-2,4-dione (24). Potassium permanganate (0.27 g) was portionwise added to a cooled (0 °C) suspension of compound **23** (0.57 mmol) in a 1:1 acetone/water mixture (10 mL). Each small addition was made after the disappearance of the violet color of the oxidizing agent. After the additions were completed, the mixture was stirred at room temperature for 1 h, then the excess of potassium permanganate was quenched with a 38% solution of sodium hydrogen sulfite, and the solution was acidified with 6 N HCl. The solid was collected, washed with water, and recrystallized. Yield: 48%; mp > 300 °C (EtOH). ^{1}H NMR: 2.40 (s, 3H, CH_3), 6.56 (s, 1H, pyrrole proton), 7.03 (s, 1H, pyrrole proton), 7.37 (s, 1H, ar), 7.56 (s, 1H, pyrrole proton), 7.89 (s, 1H, ar), 12.10 (br s, 2H, NH + COOH). Anal. ($C_{15}H_{10}ClN_3O_6$) C, H, N.

7-Chloro-3-hydroxy-6-(3-carboxypyrrol-1-yl)-1*H*-quinazoline-2,4-dione (9). A solution of the 3-acetoxy derivative **24** (0.3 mmol) in ethanol (2 mL), containing 2−3 drops of piperidine, was refluxed for 1 h. After cooling at room temperature and dilution with water (10 mL), a solid was obtained that was collected and recrystallized. Yield: 64%; mp > 300 °C (EtOH). ^{1}H NMR: 6.56 (s, 1H, pyrrole proton), 7.03 (s, 1H, pyrrole proton), 7.37 (s, 1H, H-8), 7.56 (s, 1H, pyrrole proton), 7.89 (s, 1H, H-5), 10.82 (s, 1H, OH), 11.82 (s, 1H, NH), 12.03 (br s, 1H, COOH). IR: 1700, 1740, 1750, 2500−3600. Anal. ($C_{13}H_8ClN_3O_5$) C, H, N.

3-Acetoxy-7-chloro-6-(2-oxo-2,5-dihydropyrrol-1-yl)-1*H*-quinazoline-2,4-dione (25). 2,5-Dimethoxy-2,5-dihydrofuran (1.1 mmol) and sodium acetate (2 mmol) were added to a suspension of derivative **21**[40] in glacial acetic acid (5 mL), and the mixture was heated at 90 °C for 2 h. After cooling at room temperature, the solution was diluted with water (30−40 mL), and the resulting solid was collected, washed with water, and recrystallized. Yield: 82%; mp 280 °C dec (EtOH). ^{1}H NMR: 2.39 (s, 3H, CH_3), 4.42 (s, 2H, CH_2), 6.25 (d, 1H, pyrrole proton, J = 5.9 Hz), 7.31 (s, 1H, H-8), 7.50 (d, 1H, pyrrole proton, J = 5.9 Hz), 7.97 (s, 1H, H-5), 12.15 (br s, 1H, NH). IR: 1670, 1710, 1750, 1815. Anal. ($C_{14}H_{10}ClN_3O_5$) C, H, N.

7-Chloro-3-hydroxy-6-(2-oxo-2,5-dihydropyrrol-1-yl)-1*H*-quinazoline-2,4-dione (10). The title compound was prepared from the 3-acetoxy derivative **25** (0.3 mmol) in the experimental conditions described above to obtain **9** from **24**. Yield: 82%; mp > 300 °C (EtOH). ^{1}H NMR: 4.43 (s, 2H, CH_2), 6.25 (d, 1H, pyrrole proton, J = 5.9 Hz), 7.31 (s, 1H, H-8), 7.50 (d, 1H, pyrrole proton, J = 5.9 Hz), 7.97 (s, 1H, H-5), 10.72 (br s, 1H, OH), 11.72 (br s, 1H, NH). IR: 1680, 1740, 3100−3600. Anal. ($C_{12}H_8ClN_3O_4$) C, H, N.

7-Chloro-3-hydroxy-6-(4-oxo-4*H*-pyridin-1-yl)-1*H*-quinazoline-2,4-dione (11). A solution of the 6-amino derivative **21**[40] (1.1 mmol) and 4-oxo-4*H*-pyran-2,6-dicarboxylic acid (chelidonic acid; 1.4 mmol) in dimethyl sulfoxide (2 mL) was stirred at 90 °C for 58 h. After cooling at room temperature, the solution was diluted with water (50 mL), and the solid was filtered off. The solvent of the clear solution was distilled at reduced pressure, and the oily residue was taken up with acetone (5 mL) and a few drops of water. The solid was collected and recrystallized. Yield: 15%; mp > 300 °C (AcOH). ^{1}H NMR: 6.20 (d, 2H, pyridine protons, J = 7.6 Hz), 7.41 (s, 1H, H-8), 7.73 (d, 2H, pyridine protons, J = 7.6 Hz), 8.12 (s, 1H, H-6), 10.82 (s, 1H, OH), 11.90 (s, 1H, NH). IR: 1683, 1732, 3054−3600. Anal. ($C_{13}H_8ClN_3O_4$) C, H, N.

3-Acetoxy-7-chloro-6-phthalimido-1*H*-quinazoline-2,4-dione (26). A mixture of the 6-amino derivative **21** (0.9 mmol), phthalic anhydride (1.6 mmol), and sodium acetate (0.9 mmol) in glacial acetic acid (5 mL) was heated at 60 °C for about 15 h. After cooling at room temperature, the suspension was diluted with water (30 mL) and acidified to pH 1 with 6 N HCl. The solid was collected, washed with water, and recrystallized. Yield: 65%; mp > 300 °C (CH_3NO_2). ^{1}H NMR: 2.42 (s, 3H, CH_3), 7.48 (s, 1H, H-8), 7.95−8.02 (m, 4H, ar), 8.32 (s, 1H, ar), 12.28 (br s, 1H, NH). IR: 1702, 1750, 1777, 1813, 3210. Anal. ($C_{18}H_{10}ClN_3O_6$) C, H, N.

7-Chloro-6-(2-carboxybenzoylamino)-3-hydroxy-1*H*-quinazoline-2,4-dione (12). A suspension of derivatives **26** (0.9 mmol) in 1% aqueous NaOH (15 mL) was stirred at room temperature for about 30 min. The solution was acidified to pH 1 with 6 N HCl, and the resulting solid was collected, washed with water, and recrystallized. Yield: 85%; mp > 300 °C (EtOH). ^{1}H NMR: 7.31 (s, 1H, H-8), 7.57−7.70 (m, 3H, ar), 7.91 (d, 1H, ar, J = 7.6 Hz), 8.23 (s, 1H, H-5), 10.12 (s, 1H, exchangeable with D_2O), 10.68 (s, 1H, exchangeable with D_2O), 11.65 (s, 1H, exchangeable with D_2O), 13.18 (s, 1H, exchangeable with D_2O). IR: 1619, 1688, 1733, 2400−3600. Anal. ($C_{16}H_{10}ClN_3O_6$) C, H, N.

3-Acetoxy-6-(3-carboxypropanoylamino)-7-chloro-1*H*-quinazoline-2,4-dione (27). A mixture of the 6-amino derivative **21** (1.3 mmol), succinic anhydride (1.3 mmol), and sodium acetate (1.3 mmol) in glacial acetic acid (5 mL) was heated at 60° C for about 6 h. After cooling at room temperature, the suspension was diluted with water (30 mL) and acidified to pH 1 with 6 N HCl. The solid was collected, washed with water, and recrystallized. Yield: 50%; mp 223−225 °C (EtOAc). ^{1}H NMR: 2.38 (s, 3H, CH_3), 2.48−2.63 (m, 4H, $2CH_2$), 7.30 (s, 1H, H-8), 8.22 (s, 1H, H-5), 9.72 (s, 1H, COOH), 12.00 (s, 1H, NH), 12.15 (s, 1H, NH). Anal. ($C_{14}H_{12}ClN_3O_7$) C, H, N.

7-Chloro-6-(3-carboxypropanoylamino)-3-hydroxy-1*H*-quinazoline-2,4-dione (13). The title compound was synthesized from derivative **27** (0.9 mmol), as described above to prepare **12** from **26**. Yield: 80%; mp 286−287 °C (EtOH). ^{1}H NMR: 2.47−2.61 (m, 4H, al), 7.24 (s, 1H, ar), 8.17 (s, 1H, ar), 9.67 (s, 1H, COOH), 10.65 (br s, 1H, OH), 11.80 (br s, 2H, 2NH). Anal. ($C_{12}H_{10}ClN_3O_6$) C, H, N.

3-Acetoxy-7-chloro-6-(2,5-dioxo-pyrrolidin-1-yl)-1*H*-quinazoline-2,4-dione (28). Sodium acetate (1.2 mmol) was added to a suspension of compound **27** (0.7 mmol) in acetic anhydride (8 mL). The mixture was refluxed for 2 h, the excess of acetic anhydride was evaporated at reduced pressure, and the residue was taken up with water (20 mL). The suspension was acidified to pH 1 with a few drops of 6 N HCl, and the solid was collected, washed with water, and recrystallized. Yield: 82%; mp > 300 °C (EtOH). ^{1}H NMR: 2.39 (s, 3H, CH_3), 2.82−2.84 (m, 4H, $2CH_2$), 7.40 (s, 1H, H-8), 8.06 (s, 1H, H-5), 12.2 (br s, 1H, NH). Anal. ($C_{14}H_{10}ClN_3O_6$) C, H, N.

7-Chloro-3-hydroxy-6-(2,5-dioxo-pyrrolidin-1-yl)-1*H*-quinazoline-2,4-dione (14). The title compound was prepared from the corresponding 3-acetoxy derivative **28** (0.3 mmol) as described above to prepare derivative **9** from **24**. Yield: 70%; mp > 300 °C (EtOH). ^{1}H NMR: 2.78−2.84 (m, 4H, $2CH_2$), 7.34 (s, 1H, H-8), 8.01 (s, 1H, H-5), 10.70 (br s, 1H, OH), 11.81 (br s, 1H, NH). IR: 1690, 1710, 1730, 1750, 3120−3520. Anal. ($C_{12}H_8ClN_3O_5$) C, H, N.

3-Acetoxy-6-benzoylamino-7-chloro-1*H*-quinazoline-2,4-dione (29). A solution of benzoyl chloride (1.1 mmol) in anhydrous methylene chloride (3 mL) was dropwise added to a cooled (0 °C) suspension of the 6-amino derivative **21**[40] (1.1 mmol) in anhydrous methylene chloride (10 mL) and pyridine (1 mL). When the addition was completed, the mixture was stirred at room temperature for 24 h. Evaporation of the solvent at reduced pressure gave an oily residue that was treated with water (3 mL) and ethanol (3 mL). The resulting solid was collected and recrystallized. Yield: 60%; mp 259−261 °C (EtOH). ^{1}H NMR: 2.39 (s, 3H, CH_3), 7.37 (s, 1H, ar), 7.50−7.62 (m, 3H, ar), 7.97 (d, 2H, ar, J = 7.7 Hz), 8.09 (s, 1H, ar), 10.25 (s, 1H, NH), 12.10 (br s, 1H, NH). Anal. ($C_{17}H_{12}ClN_3O_5$) C, H, N.

General Procedure to Prepare 3-Acetoxy-6-[3-(methoxycarbonyl)benzoylamino]-7-chloro-1*H*-quinazoline-2,4-dione (30) and 3-Acetoxy-6-[4-(methoxycarbonyl)benzoylamino]-7-chloro-1*H*-quinazoline-2,4-dione (31). A solution of 3-methoxycarbonylbenzoyl chloride[44] or 4-methoxycarbonylbenzoyl chloride[45] (4 mmol) in anhydrous methylene chloride (3 mL) and, in succession, a solution of anhydrous pyridine (4 mmol) in anhydrous methylene chloride (3 mL) were dropwise added, over a period of about 15 min, to a cooled (0 °C) suspension of the 6-amino derivative **21**[40] (2.1 mmol) in anhydrous methylene chloride (20 mL). The suspension was stirred at room temperature for about 10 h (compound **30**) or 7 h (compound **31**). The crude **30** was collected by filtration, resuspended in EtOAc (100 mL), and stirred for a few minutes, then it was collected, washed with water, and recrystallized. The crude **31** was collected, washed with water, and recrystallized.

Compound 30. Yield: 40%; mp > 300 °C (EtOH). ^{1}H NMR: 2.41 (s, 3H, $COCH_3$), 3.92 (s, 3H, $COOCH_3$), 7.41 (s, 1H, H-8), 7.72 (t, 1H, ar, J = 8.0 Hz), 8.11 (s, 1H, H-5), 8.19 (d, 1H, ar, J = 8.0 Hz), 8.26 (d, 1H, ar, J = 8.0 Hz), 8.58 (s, 1H, ar), 10.51 (s, 1H, NH), 12.11 (s, 1H, NH). Anal. ($C_{19}H_{14}ClN_3O_7$) C, H, N.

Compound 31. Yield: 70%; mp > 300 °C (EtOH). ^{1}H NMR: 2.41 (s, 3H, $COCH_3$), 3.91 (s, 3H, $COOCH_3$), 7.40 (s, 1H, H-8), 8.10−8.15 (m, 5H, 4 ar + H-5), 10.47 (s, 1H, NH), 12.12 (s, 1H, NH). Anal. ($C_{19}H_{14}ClN_3O_7$) C, H, N.

General Procedure to Prepare 6-Benzoylamino-7-chloro-3-hydroxy-1*H*-quinazoline-2,4-dione (15), 6-(3-Carboxybenzoylamino)-7-chloro-3-hydroxy-1*H*-quinazoline-2,4-dione (16), and 6-(4-Carboxybenzoylamino)-7-chloro-3-hydroxy-1*H*-quinazoline-2,4-dione (17). A solution of the 3-acetoxy derivative **29**, **30**, or **31** (0.8 mmol) in aqueous 2% NaOH (10 mL) was stirred at room temperature for 45 min and then acidified to pH 1 with 6 N HCl. The resulting solid was collected, washed with water, and recrystallized. Compounds **15** and **17** were recrystallized, while compound **16** was purified by acid/base exchange as follows: the crude product was dissolved in aqueous 0.4% NaOH (10 mL), and the solution was acidified to pH 1 with 6 N HCl to yield a solid that was collected by filtration.

Compound 15. Yield: 60%; mp > 300 °C (EtOH). ^{1}H NMR: 7.31 (s, 1H, ar), 7.50−7.62 (m, 3H, ar), 7.98 (d, 2H, ar, J = 7.0 Hz), 8.07 (s, 1H, ar), 10.21 (s, 1H, exchangeable with D_2O), 10.72 (br s, 1H, exchangeable with D_2O), 11.63 (br s, 1H, exchangeable with D_2O). IR: 1680, 1740, 3060−3600. Anal. ($C_{15}H_{10}ClN_3O_4$) C, H, N.

Compound 16. Yield: 87%; mp > 300 °C. ^{1}H NMR: 7.34 (s, 1H, H-8), 7.65−7.70 (m, 1H, ar), 8.07 (s, 1H, H-5), 8.17−8.21 (m, 2H, ar), 8.57 (s, 1H, ar), 10.42 (s, 1H, exchangeable with D_2O), 10.69 (s, 1H, exchangeable with D_2O), 11.68 (s, 1H, exchangeable with D_2O), 13.27 (br s, 1H, exchangeable with D_2O). IR: 1624, 1685, 1730, 2400−3600. Anal. Calcd for $C_{16}H_{10}ClN_3O_6$: C, 51.14; H, 2.69; N, 11.19. Found: C, 51.64; H, 2.24; N, 11.61.

Compound 17. Yield: 74%; mp > 300 °C (EtOH). ^{1}H NMR: 7.34 (s, 1H, H-8), 8.01−8.08 (m, 5H, 4 ar + H-5), 10.37 (s, 1H, exchangeable with D_2O), 10.70 (s, 1H, exchangeable with D_2O), 11.69 (s, 1H, exchangeable with D_2O), 13.27 (br s, 1H, exchange-

able with D_2O). IR: 1625, 1667,1748, 2400−3600. Anal. ($C_{16}H_{10}ClN_3O_6$) C, H, N.

General Procedure to Prepare 7-Chloro-3-hydroxy-6-[(3-methoxycarbonyl)benzoylamino]-1*H*-quinazoline-2,4-dione (18) and 7-Chloro-3-hydroxy-6-[(4-methoxycarbonyl)benzoylamino]-1*H*-quinazoline-2,4-dione (19). The title compounds were prepared from the corresponding 3-acetoxy derivatives **30** and **31** (0.7 mmol), as described above, to obtain compound **9** from **24**.

Compound 18. Yield: 74%; mp > 300 °C (DMF). ^{1}H NMR: 3.92 (s, 3H, CH_3), 7.32 (s, 1H, H-8), 7.72 (t, 1H, ar, J = 8.0 Hz), 8.04 (s, 1H, H-5), 8.20 (d, 1H, ar, J = 8 Hz), 8.24 (d, 1H, ar, J = 8.0 Hz), 8.58 (s, 1H, ar), 10.31 (br s, 1H, exchangeable with D_2O), 11.02 (br s, 2H, exchangeable with D_2O). Anal. ($C_{17}H_{12}ClN_3O_6$) C, H, N.

Compound 19. Yield: 58%; mp > 300 °C (2-methoxyethanol). ^{1}H NMR: 3.91 (s, 3H, CH_3), 7.32 (s, 1H, H-8), 8.05 (s, 1H, H-5), 8.11 (s, 4H, ar), 10.42 (br s, 1H, exchangeable with D_2O), 11.00 (br s, 2H, exchangeable with D_2O). Anal. ($C_{17}H_{12}ClN_3O_6$) C, H, N.

3-Acetoxy-6-(3-benzylureido)-7-chloro-1*H*-quinazoline-2,4-dione (32). A mixture of the 6-amino derivative **21**[40] (1.1 mmol) and benzylisocyanate (1.7 mmol) in anhydrous tetrahydrofuran (15 mL) was refluxed for 5 h under a nitrogen atmosphere. The suspension was cooled at room temperature and diluted with water (40 mL). The solid was collected, washed with water, and recrystallized. Yield: 90%; mp > 300 °C (EtOH). ^{1}H NMR: 2.40 (s, 3H, CH_3), 4.33 (d, 2H, CH_2), 7.25−7.48 (m, 7H, ar), 8.29 (s, 1H, NH), 8.72 (s, 1H, NH), 11.88 (s, 1H, NH). IR: 1680, 1748, 1814, 3270. Anal. ($C_{18}H_{15}ClN_4O_5$) C, H, N.

6-(3-Benzylureido)-7-chloro-3-hydroxy-1*H*-quinazoline-2,4-dione (20). The title compound was prepared from the corresponding 3-acetoxy derivative **32** (0.7 mmol) in the conditions described above to obtain **12** from **26**. Yield: 80%; mp > 300 °C (MeOH). ^{1}H NMR: 4.33 (s, 2H, CH_2), 7.33−7.42 (m, 7H, ar), 8.21 (s, 1H, NH), 8.69 (s, 1H, NH), 10.59 (s, 1H, OH), 11.45 (s, 1H, NH). IR: 1677, 1746, 3100−3500. Anal. ($C_{16}H_{13}ClN_4O_4$) C, H, N.

Pharmacology. Binding Assays. Rat cortical synaptic membrane preparation and [^{3}H]glycine, [^{3}H]AMPA binding experiments were performed following the procedure reported in refs 34 and 57, respectively. The high-affinity and low-affinity [^{3}H]kainate binding assays were performed on rat cortical membranes according to previously described methods.[41]

Electrophysiological Assays. The mouse cortical wedge preparation described by Mannaioni et al.[58] was used, while the electrophysiological assays were performed following the procedures described in ref 38.

Sample Preparation and Result Calculation. A stock 1 mM solution of the tested compound was prepared in 50% DMSO, and the subsequent dilution was 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.[59]

Pharmacological Assays. Abdominal Constriction Test. Mice were injected ip with a 0.6% solution of acetic acid (10 mL kg^{-1}), according to Koster et al.[60] The number of stretching movements was counted for 10 min, starting 5 min after acetic acid injection.

Rota-Rod Test. The apparatus consisted of a base platform and a rotating rod of 3-cm diameter, with a nonslippery surface. The rod was placed at a height of 15 cm from the base. The rod, 30 cm in length, was divided into five equal sections by six disks. Thus, up to five mice were tested simultaneously on the apparatus, with a rod-rotating speed of 16 rpm. The integrity of motor coordination was assessed on the basis of the number of falls from the rod in 30 s, according to Vaught et al.[61] The performance time was measured before treatment and 30, 45, and 60 min after treatment.

Administration of Drug. Compound **12** was dispersed in sodium carboxymethylcellulose (CMC) 1% immediately before use. Drug concentrations were prepared in such a way that the necessary dose could be administered in a volume of 10 mL kg^{-1} by per os (po) route 30 min before tests.

Molecular Modeling. Compounds were modeled using the LigPrep Schrodinger ligands preparation procedure (pH 7.4) and minimized with Macromodel8.5[62]

The experimental crystal structure of GluR2S1S2J/ATPO complex (PDB entry: 1n0t) and a homology-built model of GluR5/ATPO were used in the docking computation. Docking calculations were performed using the Glide program (FirstDiscovery v3.0, Schrödinger)[63] on an AMD Athlon 2800 processor running Linux. The crystal structure was prepared according to the protein preparation procedure recommended. Default input parameters were used in all computations (no scaling factor for the vdW radii of nonpolar protein atoms, 0.8 scaling factor for nonpolar ligand atoms). Upon completion of each docking calculation, three poses per ligand were saved. The best-docked structure was chosen using a model energy score (Emodel) derived from a combination of the glide score (Gscore, a modified and extended version of the empirically based ChemScore function[64]), coulombic, and the van der Waals energies and the internal strain energy of the ligands.[63]

Molecular docking of all the compounds on the Gly/NMDA receptor was carried out according to guidelines outlined in ref 42.

A comparative model of GluR5/ATPO was built by means of the Prime Protein Structure Prediction Suite (Schrodinger)[65] that consists of the prime-structure prediction and prime refinement options. The GluR5/glutamate (**1ycj**, chain A) was used as query sequence, and both GluR2/ATPO (**1n0t**, chain A) and GluR2/DNQX (**1ftl**, chain A) were used as template sequences. This made an alignment based both on sequence and on secondary structure information possible (prime align tool). The GluR5 3D model structure was obtained through the build structure model tool, and structural analysis of the homology-built model was carried out with both the prime refinement tool program ProSa2003[66] and Whatcheck.[67]

Supporting Information Available: Combustion analysis data of the newly synthesized compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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