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Novel AMPA and kainate receptor antagonists containing the pyrazolo[1,5-*c*]quinazoline ring system: Synthesis and structure–activity relationships

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Abstract—This paper reports the synthesis and AMPA, Gly/NMDA, and KA receptor binding affinities of a new set of 1,9-disubstituted-8-chloro-pyrazolo[1,5-*c*]quinazoline-2-carboxylates **2**–**34**. Binding data show that, in general, compounds **2**–**34** bind to the AMPA receptor with good affinity and selectivity. In particular, the obtained results indicate that the contemporary presence of a 1,2-dicarboxylic acid moiety and suitable benzo-substituents on the PQZ system is important to gain selective AMPA receptor antagonists. Moreover, this study shows that the presence of a 2-carboxybenzoylamino substituent at position-9 (compounds **33**–**34**) is important for obtaining selective KA receptor antagonists. Some selected compounds were also tested for their functional antagonistic activity at both AMPA and NMDA receptor-ion channels.

1. Introduction

Glutamic acid (Glu) is the major excitatory neurotransmitter in the central nervous system (CNS). The effects of Glu are mediated by ionotropic (iGluRs) and metabotropic receptors (mGluRs).[1]

The iGluRs have been divided into three main groups: NMDA (*N*-methyl-D-aspartic acid), AMPA (2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)-propionic acid), and KA (kainic acid) receptors, based on their pharmacology, and more recently on their amino acid sequence homology.[1,2] The NMDA receptor complex possesses different binding sites including the glycine coagonist receptor (Gly/NMDA).[3]

It is generally agreed that iGluRs are implicated in a variety of CNS functions including learning and memory. However, over-stimulation of postsynaptic iGluRs is known to worsen neuro-degeneration in conditions such as hypoxia/ischemia, brain damage,[4] epilepsy,[1] Parkinson's[5] and Alzheimer's diseases,[6] and multiple sclerosis.[7] Accordingly, there is great interest for competitive iGluR antagonists as well as for non-competitive antagonists acting at the Gly/NMDA receptor for their potential therapeutic use in the above mentioned pathologies.[1,8,9]

Availability of selective Gly/NMDA and AMPA receptor agonists and antagonists has made it possible to clearly establish both the physiological and pathophysiological role of Gly/NMDA and AMPA receptors.[1,8,9] In contrast, knowledge about the role of KA receptors is still in its early stages since only recently were some selective KA receptor antagonists reported.[10–15]

In the last few years in our laboratory we prepared two series of 5,6-dihydro-pyrazolo[1,5-*c*]quinazoline-2-carboxylate (PQZ), **1A** and **1B** (Chart 1), as Gly/NMDA and/or AMPA receptor antagonists.[16,17]

Structure–activity relationship (SAR) studies on series **1A** have highlighted that the contemporary presence of a chlorine atom and a nitrogen-containing heterocycle at position-8 and -9, respectively, shifted affinity and

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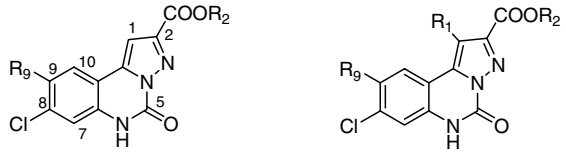


1A **1B**

R_2 = H, Et

R_9 = H, Cl, NO_2 heterocycles

R_1 = COOEt, COOH, Cl, Br, CH_3

R_9 = H, Cl

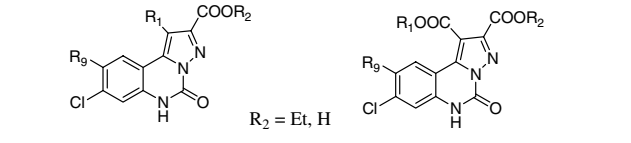
Chart 1. Previously reported PQZ derivatives.

selectivity of PQZ derivatives toward the AMPA receptor.[16] Furthermore, even if PQZ derivatives show lower affinities for the KA receptor than for the AMPA one, the SARs of these two receptors are comparable, thus confirming the high structural similarity of their binding pockets.[16]

Further studies on PQZ derivatives, also supported by molecular modeling investigations, pointed out that the contemporary presence of substituents on the fused benzo ring and at position-1 seems to affect both potency and receptor selectivity (series **1B**).[17]

As a continuation of our research, we report in the present paper the synthesis of two new sets of 1,9-disubstituted-8-chloro-pyrazolo[1,5-*c*]quinazoline-2-carboxylates **2**–**13** and **14**–**34** (Chart 2) designed as AMPA and/or KA receptor antagonists.

Compounds **2**–**13** were synthesized in order to identify the best R_1 group to enhance affinity and selectivity toward the AMPA receptor. In fact, derivatives **2**–**13** bear diverse substituents at position-1 (Cl, NO_2, COOEt, COOH) and at position-9 display substituents (NO_2, heterocycles) which are well known to enhance AMPA receptor affinity and selectivity in many classes of AMPA receptor antagonists.[8,16,18–20] Compounds **14**–**32** were designed holding the 1-carboxylate function constant and by introducing at position-9 some ureido moieties which we did not investigate in our previously reported PQZ derivatives. Finally, the synthesis of the 9-(2-carboxybenzoylamino) derivatives **33** and **34**, thought to be selective KA receptor antagonists, was performed. In fact, the 2-carboxybenzoylamino substituent introduced in a similar position on the fused benzo ring of our 7-chloro-3-hydroxy-quinazoline-2,4-dione scaffold afforded a selective KA receptor antagonist.[15]



R_2 = Et, H

2-13: R_1 = Cl, NO_2, COOEt, COOH; R_9 = NO_2, heterocycles

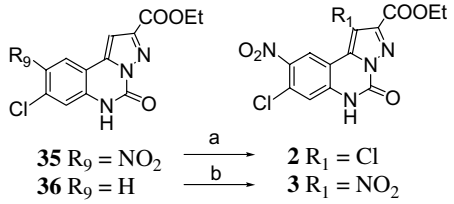
14-32: R_1 = Et, H; R_9 = NHCONHR

33-34: R_1 = Et, H; R_9 = NHCO-(2-$COOHC_6H_5$)

Chart 2. Currently reported PQZ derivatives.

2. Chemistry

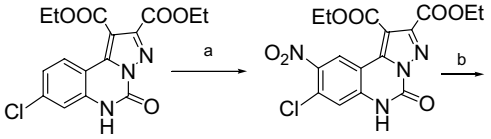
Compounds **2**–**34** were synthesized as depicted in Schemes 1–4. Briefly, the 1-chloro-9-nitro-2-ethyl ester **2**[21] (Scheme 1) was obtained by chlorination of the corresponding 1-unsubstituted-9-nitro ester **35**,[16] while the 1,9-dinitro-2-ethyl ester **3** was prepared by nitration of the known ethyl 8-chloro-5,6-dihydro-5-oxo-pyrazolo[1,5-*c*]quinazoline-2-carboxylate **36**.[16]



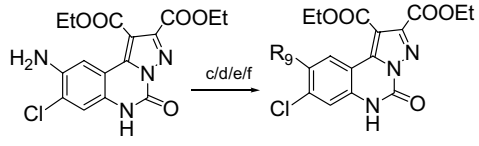
35 R_9 = NO_2 —a→ **2** R_1 = Cl

36 R_9 = H —b→ **3** R_1 = NO_2

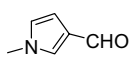
Scheme 1. Reagents and conditions: (a) SO_2Cl_2, glacial AcOH, I_2, 50 °C; (b) 90% HNO_3, 0 °C to room temperature.



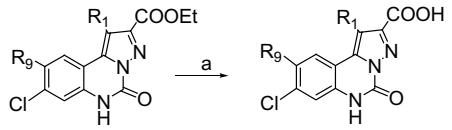
37 **4**



38 **5-7, 14-23**

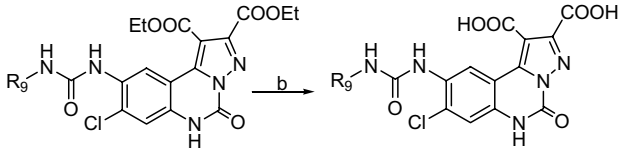
n°	R_9	n°	R_9
5		18	$NHCONHC_6H_5$
6		19	$NHCONHCH_2C_6H_5$
7		20	$NHCONHCH_2CH_2C_6H_5$
14	$NHCONHCH_2COOEt$	21	$NHCONH(3\text{-}COOEtC_6H_4)$
15	$NHCONHCH_2CH_2COOEt$	22	$NHCONH(4\text{-}COOEtC_6H_4)$
16	$NHCONHCH_2CH_2CH_2COOEt$	23	$NHCONHCH(COOMe)CH_2C_6H_5$
17	$NHCONHCH_2CH_3$		

Scheme 2. Reagents and conditions: (a) 90% HNO_3, 0 °C; (b) iron powder, glacial AcOH, reflux; (c) diformylhydrazine, Me_3SiCl, Et_3N, pyridine, reflux; (d) 2,5-diethoxytetrahydrofuran, glacial AcOH, 90 °C; (e) 2,5-dimethoxy-3-tetrahydrofurancarboxyaldehyde, glacial AcOH, 90 °C; (f) RNCO, tetrahydrofuran, 70 °C.



2-6 **8-13**

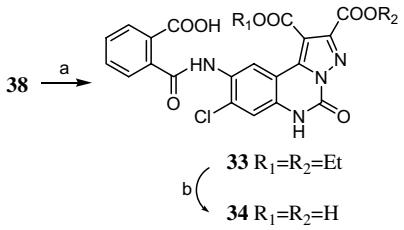
entry	R_9	R_1	method	n°	R_1
2	NO_2	Cl	A	8	Cl
3	NO_2	NO_2	A	9	NO_2
4	NO_2	COOEt	B	10	COOEt
4	NO_2	COOEt	C	11	COOH
5		COOEt	C	12	COOH
6		COOEt	D	13	COOH



14-15, 17-23 **24-32**

entry	R_9	n°	R_9
14	CH_2COOEt	24	CH_2COOH
15	CH_2CH_2COOEt	25	CH_2CH_2COOH
17	CH_2CH_3	26	CH_2CH_3
18	C_6H_5	27	C_6H_5
19	$CH_2C_6H_5$	28	$CH_2C_6H_5$
20	$CH_2CH_2C_6H_5$	29	$CH_2CH_2C_6H_5$
21	3-$COOEtC_6H_4$	30	3-$COOHC_6H_4$
22	4-$COOEtHC_6H_4$	31	4-$COOHC_6H_4$
23	$CH(COOMe)CH_2C_6H_5$	32	$CH(COOH)CH_2C_6H_5$

Scheme 3. Reagents and conditions: (a) Method A: i—10% KOH, MeOH, reflux; ii—6 N HCl; Method B: i—10% KOH, MeOH, room temperature; ii—12 N HCl; Method C: i—10% KOH, EtOH, reflux; ii—12 N HCl; Method D: i—1 N NaOH, room temperature; ii—6 N HCl; (b) Method E: i—10% NaOH, EtOH, reflux; ii—6 N HCl.



Scheme 4. Reagents and conditions: (a) phthalic anhydride, NaOAc, glacial AcOH, 60 °C; (b) i—1 N NaOH, room temperature; ii—6 N HCl.

In Scheme 2, the synthesis of the ethyl 1,2-dicarboxylates **4**–**7** and **14**–**23** is reported.

Allowing the known ethyl 8-chloro-1,2-dicarboxylate **37**[17] to react with 90% nitric acid, the corresponding 9-nitro derivative **4** was obtained, which was reduced to afford the corresponding 9-amino derivative **38**. By reacting the latter either with 1,2-diformylhydrazine or 2,5-diethoxytetrahydrofuran, the 9-(1,2,4-triazol-4-yl) ester **5** and the 9-(pyrrol-1-yl) ester **6**, respectively, were obtained. Reaction of **38** with an excess of 2,5-dimethoxy-3-tetrahydrofurancarboxaldehyde yielded the 9-(3-formylpyrrol-1-yl) derivative **7**. Finally, reaction of the 9-amino **38** with an excess of suitable isocyanates afforded the corresponding 9-ureido derivatives **14**–**23**.

Alkaline hydrolysis of the esters **2**–**6**, **14**–**15**, and **17**–**23** carried out at room temperature or at reflux, yielded acids **8**–**13**[21] and **24**–**32** (Scheme 3).

Finally, reaction of the 9-amino derivative **38** with phthalic anhydride afforded the 9-(2-carboxybenzoylamino)-1,2-diethyl ester **33** that yielded the corresponding 1,2-dicarboxylic acid **34** by alkaline hydrolysis (Scheme 4).

3. Results and discussion

The pyrazoloquinazolines **2**–**34** were evaluated for their binding at AMPA, Gly/NMDA, and KA receptors and the results are listed in Tables 1–3. In Table 1 the binding data of the previously reported 1-unsubstituted derivative **1a** are also included as references.

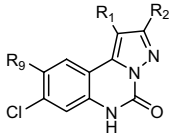
Compounds **2**–**13** (Table 1) were synthesized in order to identify the best R_1 substituent to enhance affinity and/or selectivity toward the AMPA receptor.

To reach our goal, we first synthesized the 9-nitro-2-ethyl esters **2**–**4** and their corresponding 2-carboxylic acids **8**–**11** bearing diverse substituents (Cl, NO_2, COOEt, COOH) at position-1. The binding data show that acids **8**–**11** are more active toward the AMPA and Gly/NMDA receptors than their corresponding esters **2**–**4**. These results are in accordance with our previously reported molecular docking studies which showed that the presence of a carboxylic acid group at position-2 of the PQZ system is important for the binding to AMPA and Gly/NMDA receptors due to an electrostatic interaction between the negatively charged carboxylate group and a cationic site on the receptors.[17]

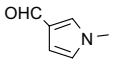
Among the 2 carboxylic acids **8**–**11**, the highest AMPA receptor affinity appears when a carboxylic acid group is present at position-1 (see compound **11**). This could be rationalized on the basis of our previous docking investigations[17] which clearly indicated that the AMPA site environment around the R_1 position is a polar region which better accommodates hydrophilic groups like carboxylic acids rather than lipophilic moieties like a chlorine atom, a nitro group, or an ethyl carboxylate group (compare **11** with **8**–**10**).

Replacement of the 9-nitro group of 1,2-diethyl ester **4** and of its corresponding acid **11** with heterocycle moieties yields esters **5**–**7** and acids **12**–**13**. The positive effect exerted by the presence of a 9-heterocycle group is clearly highlighted by the ameliorated AMPA receptor binding affinity of esters **5**–**7** with respect to their parent compound **4**. In particular, the 9-(1,2,4-triazol-4-yl) derivative **5** shows a micromolar AMPA receptor affin-

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



1a, 2-13

Compound	R_1	R_2	R_9	K_i[a] (μM) or I%[b]		AMPA versus Gly/NMDA selectivity ratio	IC_{50}[c] (μM) or I%[b]
				[^{3}H]AMPA	[^{3}H]glycine		[^{3}H]KA
1a[d]	H	COOH		0.14 ± 0.02	8.3 ± 2.0	59.3	5.1 ± 1.4
2	Cl	COOEt	NO_2	63 ± 7	45%	>1.5	25%
3	NO_2	COOEt	NO_2	45%	36.2 ± 3.1	0.4	16%
4	COOEt	COOEt	NO_2	40%	30%		0%
5	COOEt	COOEt		1.2 ± 0.2	18%	>80	82 ± 9
6	COOEt	COOEt		48 ± 6	13%	>2	12%
7	COOEt	COOEt		15.7 ± 2.1	20%	>6	17%
8	Cl	COOH	NO_2	1.3 ± 0.1	2.5 ± 0.1	1.9	94 ± 7
9	NO_2	COOH	NO_2	11.9 ± 3.5	20.5 ± 6.0	1.7	29%
10	COOEt	COOH	NO_2	0.94 ± 0.07	4.7 ± 0.4	5	60 ± 5
11	COOH	COOH	NO_2	0.59 ± 0.11	1.25 ± 0.25	2	11.7 ± 2.1
12	COOH	COOH		0.1 ± 0.02	43%	>1000	2.4 ± 0.7
13	COOH	COOH		0.13 ± 0.03	20 ± 3	153.8	4.5 ± 0.3

[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] Ref. 16.

ity and also a notable AMPA versus Gly/NMDA receptor selectivity.

The binding data of acids **12**–**13** are even more interesting. They show somewhat improved AMPA receptor affinities with respect to the parent acid **11** and a considerable increase in AMPA versus Gly/NMDA receptor selectivity. It is worth noting that the 9-(1,2,4-triazol-4-yl) derivative **12** and the 9-(pyrrol-1-yl) derivative **13** are equipotent to the reference compound **1a**[16] at the AMPA receptor, but they are much more AMPA versus Gly/NMDA selective. Indeed, compound **12** (K_i = 0.1 μM; selectivity ratio > 1000) shows the highest affinity and AMPA versus Gly/NMDA receptor selectivity among PQZ derivatives reported till now.

Table 2. Functional antagonism at AMPA and NMDA sites

Compound	IC_{50}[a] (μM)	
	AMPA	NMDA
5	5.8 ± 0.7	>>20
12	2.5 ± 0.3	>10
1a[b]	0.5 ± 0.07	20 ± 2.0

[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] Ref. 16.

Compounds **5** and **12** were tested to evaluate their antagonistic activity by assessing their ability to inhibit depolarization induced by 5 μM AMPA and NMDA in cortical wedge preparations.[19] The results obtained from these functional antagonism studies are reported in Table 2. Compounds **5** and **12** inhibit AMPA and NMDA response in a reversible manner and their inhibitory activities are in agreement with their binding affinities.

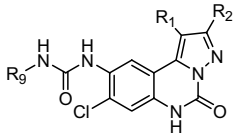
Regarding KA receptor binding activities of **2**–**13**, the data reported in Table 1 show that the presence of a carboxylic acid group at position-2 is important for receptor-ligand interaction. In fact, while the 2-ethyl esters **2**–**7** are inactive or scarcely active, the corresponding acids **8**–**13** display some KA receptor affinity, the only exception being compound **9**.

Moreover, the presence of a carboxylic acid group at position-1 is advantageous for the binding to KA receptor. Indeed, among the 2 carboxylic acids **8**–**13**, the 1,2-dicarboxylic acids **11**–**13** are endowed with the highest KA receptor affinity.

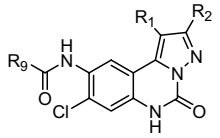
Thus, keeping the 1,2-dicarboxylate substitution pattern constant, we synthesized a new set of PQZ derivatives **14**–**32** (Table 3) bearing at position-9 diverse ureido moieties which were not investigated in our previously reported AMPA/KA receptor antagonists of similar size and shape.[16–20]

Interestingly, the results indicate that **14**–**32** are in general more active toward the AMPA receptor than toward the Gly/NMDA one. Indeed, 1,2-dicarboxylic acids **24**–**32** show submicromolar AMPA receptor binding activities and noteworthy AMPA versus Gly/NMDA receptor selectivities, compound **25** being the most active and selective (K_i = 0.16 μM; selectivity ratio = 425).

Table 3. Binding affinity at AMPA, Gly/NMDA, and KA receptors



14-32



33-34

Compound	R_1	R_2	R_9	K_i[a] (μM) or I%[b]		AMPA versus Gly/NMDA selectivity ratio	IC_{50}[c] (μM) or I%[b]
				[^{3}H]AMPA	[^{3}H]glycine		[^{3}H]KA
14	COOEt	COOEt	CH_2COOEt	8.9 ± 2.1	43%	>11	19%
15	COOEt	COOEt	CH_2CH_2COOEt	3.8 ± 0.5	32%	>26	32%
16	COOEt	COOEt	$CH_2CH_2CH_2COOEt$	3.5 ± 0.4	99 ± 9.0	28	24%
17	COOEt	COOEt	CH_2CH_3	27 ± 4	44%	>3.7	13%
18	COOEt	COOEt	C_6H_5	20%	11%	—	0%
19	COOEt	COOEt	$CH_2C_6H_5$	44%	19%	—	0%
20	COOEt	COOEt	$CH_2CH_2C_6H_5$	44%	49 ± 4	0.49	9%
21	COOEt	COOEt	3-$COOEtC_6H_4$	18%	0%	—	0%
22	COOEt	COOEt	4-$COOEtC_6H_4$	5%	5%	—	15%
23	COOEt	COOEt	$CH(COOMe)CH_2C_6H_5$	46%	16%	—	16%
24	COOH	COOH	CH_2COOH	0.48 ± 0.02	54 ± 6	112.5	4.4 ± 0.08
25	COOH	COOH	CH_2CH_2COOH	0.16 ± 0.04	68 ± 7.0	425	4.6 ± 0.2
26	COOH	COOH	CH_2CH_3	0.56 ± 0.03	41 ± 4	73.3	9.8 ± 1.1
27	COOH	COOH	C_6H_5	0.14 ± 0.02	11.7 ± 2.5	83.5	11 ± 1
28	COOH	COOH	$CH_2C_6H_5$	0.37 ± 0.04	16.3 ± 2.7	44	11 ± 2
29	COOH	COOH	$CH_2CH_2C_6H_5$	0.48 ± 0.06	31 ± 4	31	17 ± 2
30	COOH	COOH	3-$COOHC_6H_4$	0.42 ± 0.04	35 ± 3	83	3 ± 0.2
31	COOH	COOH	4-$COOHC_6H_4$	1.1 ± 0.2	49 ± 6	44	13 ± 2.0
32	COOH	COOH	$CH(COOH)CH_2C_6H_5$	0.47 ± 0.06	61 ± 6	129	40 ± 5
33	COOEt	COOEt	2-$COOHC_6H_4$	2.8 ± 0.4	36 ± 4	12.8	0.11 ± 0.02
34	COOH	COOH	2-$COOHC_6H_4$	1.3 ± 0.1	27 ± 3	20.7	0.52 ± 0.09

[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.

In particular, acids **24**–**32** are more active than their corresponding esters **14**–**23**, indicating that the 1,2-dicarboxylic acid moiety is of paramount importance for anchoring to the AMPA receptor.

Since acids **24**–**32** show comparable and good AMPA receptor binding activities, we can deduce that R_9 substituents with different electronic and steric properties are similarly well tolerated by the receptor site. In fact, AMPA receptor affinities of **24** and **25**, which bear a hydrophilic R_9 carboxyalkyl chain on the 9-ureido, match those of **28**, **29**, and **27**, respectively, which bear lipophilic and bulky R_9 ureido substituents.

Introduction of a carboxylic acid group in *meta* or *para* on the 9-phenylureido moiety of **27** yields derivatives **30** and **31**, respectively, which show comparable or slightly reduced AMPA receptor affinities with respect to **27**. Moreover, introduction of a carboxylic acid group on the ethyl chain of the 9-(phenylethyl)ureido derivative **29** afforded compound **32** which results equipotent to **29**.

Concerning AMPA versus Gly/NMDA receptor selectivity of **24**–**32**, the results are difficult to rationalize since all derivatives show comparable and good AMPA receptor selectivity apart from the nature of the R_9 substituent on the 9-ureido group. However, compounds with AMPA versus Gly/NMDA receptor selectivity ratio > 100 bear a R_9 carboxy alkyl chain (see compounds **24**, **25**, and **32**). These data lead us to hypothesize that a R_9 hydrophilic substituent, such as the carboxyalkyl one, can shift selectivity toward the AMPA receptor. Indeed, previous molecular modeling studies pointed out the hydrophilic nature of the AMPA receptor binding pocket which accommodates the R_9 substituent.[17]

Analysing KA receptor binding activities of 9-ureides **14**–**32**, the data reported in Table 3 show that the 1,2-dicarboxylic acids **24**–**32** display affinity for the KA receptor in the micromolar range with the only exception being compound **32**. In contrast, their corresponding esters **14**–**23** are inactive, suggesting the importance of the 1,2-dicarboxylic acid residue for anchoring to the KA receptor, and also indicating that ureido moieties, with different steric and electronic properties, are similarly tolerated by the receptor. As in the set of compounds **2**–**13**, the SARs at the KA receptor are similar to those at the AMPA one, and none of the acids **24**–**32** show KA versus AMPA receptor selectivity.

However, while we were obtaining the pyrazoloquinazolines **2**–**32**, the 6-(2-carboxybenzoylamino)-3-hydroxy-1*H*-quinazolin-2,4-dione was synthesized in our laboratory. This compound possesses good affinity for KA receptor and also good selectivity not only versus the Gly/NMDA receptor but also, and most importantly, versus the AMPA one.[15] On this basis, we synthesized the 1,2-dicarboxylate derivatives **33** and **34** bearing the 2-carboxybenzoylamino group at position-9, which resembles the 6 position of our quinazoline-dione lead. As expected, **33** and **34** show very interesting pharmacological profiles. In fact, with KA receptor affinities in the submicromolar range (**33**: $IC_{50} = 0.11\ \mu M$; **34**: $IC_{50} = 0.52\ \mu M$), they are the most active compounds among the PQZs reported till now. Moreover, it is worth noting that compound **33** shows KA receptor selectivity both versus Gly/NMDA and, most importantly, versus AMPA receptors.

4. Conclusions

The present study has produced new 1,9-disubstituted-8-chloro-pyrazolo[1,5-*c*]quinazolines which are endowed with good affinity and improved selectivity toward the AMPA receptor. As a new result, SAR studies have pointed out that the contemporary presence of 1,2-dicarboxylic acid moiety and suitable benzo substituents is important to enhance AMPA receptor selectivity. In particular, among PQZ derivatives reported till now, the 9-(1,2,4-triazol-4-yl)-1,2-dicarboxylic acid derivative **12** shows the highest affinity ($K_i = 0.1\ \mu M$) and AMPA versus Gly/NMDA receptor selectivity (selectivity ratio > 1000). Moreover, the new 9-ureido-1,2-dicarboxylic acid derivatives **24**–**32** display good AMPA receptor affinities ($0.14\ \mu M \leqslant K_i \leqslant 1.1\ \mu M$) and noteworthy AMPA versus Gly/NMDA receptor selectivities. Indeed, the 9-(carboxypropionic acid)-ureido derivative **25** shows high AMPA receptor affinity ($K_i = 0.14\ \mu M$) and improved AMPA versus Gly/NMDA receptor selectivity (selectivity ratio = 425) when compared with previously reported PQZ series.

However, the most significant result is the finding that the 2-carboxybenzoylamino substituent at the 9-position of the tricyclic PQZ scaffold is able to shift affinity and selectivity toward the KA receptor. Indeed, both the 9-(2-carboxybenzoylamino) derivatives **33** ($IC_{50} = 0.11\ \mu M$) and **34** ($IC_{50} = 0.52\ \mu M$) display good KA receptor affinity, and compound **33** also possesses KA receptor selectivity both *versus* the Gly/NMDA and, even more importantly, versus the AMPA receptors.

5. Experimental

5.1. Chemistry

Silica gel plates (Merck F254) 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 the results were within ±0.4% of the theoretical values 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 ^1H 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, which is always DMSO-d_6 unless otherwise stated. All the exchangeable protons were confirmed by addition of D_2O. The following abbreviations are used: s, singlet; d ,doublet; dd, double doublet; t, triplet; m, multiplet; br, broad; ar, aromatic protons;

al, aliphatic protons. The physical and analytical data of the newly synthesized compounds are shown in Table 4.

5.1.1. Synthesis of ethyl 1,8-dichloro-5,6-dihydro-9-nitro-5-oxo-pyrazolo[1,5-*c*]quinazoline-2-carboxylate (2).[21] To a suspension of ethyl 8-chloro-5,6-dihydro-9-nitro -5-oxo-pyrazolo[1,5-*c*]quinazoline-2-carboxylate **35**[16] (1.2 mmol) in glacial acetic acid (10 mL), sulfuryl chloride (2.4 mmol) and a crystal of iodine as catalyst were added. The reaction mixture was heated at 60 °C for 3 h, and then cooled at room temperature. The solid was collected by filtration and washed with water. ^{1}H NMR 1.36 (t, 3H, al, J = 7.3 Hz), 4.40 (q, 2H, al, J = 7.3 Hz), 7.54 (s, 1H, ar), 9.07 (s, 1H, ar), 12.77 (br s, 1H, NH).

Table 4. Physical and analytical data of compounds **2**–**34** and **38**

Compound	Mp (°C)	Solv.[a]	Yield (%)	C, H, N
2	292–295	A	70	$C_{13}H_8Cl_2N_4O_5$
3	191–194	B	75	$C_{13}H_8ClN_5O_7$
4	294–296	C	90	$C_{16}H_{13}ClN_4O_7$
5	>300	D	80	$C_{18}H_{15}ClN_6O_5$
6	>300	C	80	$C_{20}H_{17}ClN_4O_5$
7	>300	A	50	$C_{21}H_{17}ClN_4O_6$
8	>300	E	85	$C_{11}H_4Cl_2N_4O_5$
9	286–288	E	70	$C_{11}H_4ClN_5O_7$
10	248–250	C	60	$C_{14}H_9ClN_4O_7$
11	>300	F	80	$C_{12}H_5ClN_4O_7$
12	>300	A	80	$C_{14}H_7ClN_6O_5$
13	>300	G	55	$C_{16}H_9ClN_4O_5$
14	>300	C	65	$C_{21}H_{22}ClN_5O_8$
15	>300	C	50	$C_{22}H_{24}ClN_5O_8$
16	238–240	C	55	$C_{23}H_{26}ClN_5O_8$
17	>300	C	60	$C_{19}H_{20}ClN_5O_6$
18	>300	H	75	$C_{23}H_{20}ClN_5O_6$
19	>300	C	50	$C_{24}H_{22}ClN_5O_6$
20	>300	C	50	$C_{25}H_{24}ClN_5O_6$
21	>300	C	70	$C_{26}H_{24}ClN_5O_8$
22	>300	C	78	$C_{26}H_{24}ClN_5O_8$
23	>300	C	60	$C_{27}H_{26}ClN_5O_8$
24	>300	G	60	$C_{15}H_{10}ClN_5O_8$
25	>300	G	70	$C_{16}H_{12}ClN_5O_8$
26	>300	G	70	$C_{15}H_{12}ClN_5O_6$
27	>300	G	65	$C_{19}H_{12}ClN_5O_6$
28	>300	G	50	$C_{20}H_{14}ClN_5O_6$
29	>300	G	45	$C_{21}H_{16}ClN_5O_6$
30	>300	G	90	$C_{20}H_{12}ClN_5O_8$
31	>300	G	65	$C_{20}H_{12}ClN_5O_8$
32	>300	G	55	$C_{22}H_{16}ClN_5O_8$
33	>300	I	70	$C_{24}H_{19}ClN_4O_8$
34	>300	G	60	$C_{20}H_{11}ClN_4O_8$
38	>300	C	70	$C_{16}H_{15}ClN_4O_5$

[a] Recrystallization solvents: A, glacial acetic acid; B, toluene; C, ethanol; D, dimethylformamide; E, dimethylformamide/water; F, glacial acetic acid/dimethylformamide; G, the title compound was dissolved in the minimal amount of 1 N NaOH, the insoluble material was filtered off, and the resulting clear solution acidified with 1 N HCl; the resulting solid was collected, treated with boiling ethanol, collected again, and washed with fresh ethanol; H, acetone; I, the title compound material was dissolved in the minimal amount of $NaHCO_3$ saturated solution, the insoluble was filtered off, and the resulting clear solution acidified with 6 N HCl; the resulting solid was collected, treated with boiling ethanol, collected again, and washed with fresh ethanol.

5.1.2. Synthesis of ethyl 8-chloro-5,6-dihydro-1,9-dinitro-5-oxo-pyrazolo[1,5-*c*]quinazoline-2-carboxylate (3). Ethyl 8-chloro-5,6-dihydro-5-oxo-pyrazolo[1,5-*c*]quinazoline-2-carboxylate **36**[16] (1.03 mmol) was portionwise added to cooled (0–5 °C) HNO_3 (90%, 4 mL). The reaction mixture was stirred at room temperature for 48 h. The solution was poured onto ice and the resulting solid was collected and washed with water. ^{1}H NMR 1.34 (t, 3H, al, J = 7.2 Hz), 4.46 (q, 2H, al, J = 7.2 Hz), 7.61 (s, 1H, ar), 9.32 (s, 1H, ar), 13.21 (br s, 1H, NH). IR 1740, 3200, 3420.

5.1.3. Synthesis of diethyl 8-chloro-5,6-dihydro-9-nitro-5-oxo-pyrazolo[1,5-*c*]quinazoline-1,2-dicarboxylate (4). Diethyl 8-chloro-5,6-dihydro-5-oxo-pyrazolo[1,5-*c*]quinazoline-1,2-dicarboxylate **37**[17] (2.75 mmol) was portionwise added to cooled (0–5 °C) HNO_3 (90%, 10 mL). The reaction mixture was stirred at 0–5 °C for 20 min and then poured onto ice. The solid was collected and washed with water. ^{1}H NMR 1.30–1.36 (m, 6H, al), 4.36–4.44 (m, 4H, al), 7.58 (s, 1H, ar), 9.64 (s, 1H, ar), 12.92 (br s, 1H, NH).

5.1.4. Synthesis of diethyl 9-amino-8-chloro-5,6-dihydro-5-oxo-pyrazolo[1,5-*c*]quinazoline-1,2-dicarboxylate (38). To a hot (90 °C) solution of **4** (2.4 mmol) in glacial acetic acid (20 mL), iron powder (2.4 g) was added. The mixture was refluxed for 30 min and then evaporation at reduced pressure of the solvent afforded a solid residue that was dried and extracted in Soxhlet for one day with acetone (500 mL). The resulting solution was filtered through SiO_2. Evaporation at reduced pressure of the solvent yielded a solid that was treated with diethyl ether and collected. ^{1}H NMR 1.31–1.35 (m, 6H, al), 4.37–4.42 (m, 4H, al), 5.63 (s, 2H, NH_2), 7.28 (s, 1H, ar), 7.70 (s, 1H, ar), 12.03 (br s, 1H, NH). IR 1711, 1752, 3368, 3465.

5.1.5. Synthesis of diethyl 8-chloro-5,6-dihydro-5-oxo-9-(1,2,4-triazol-4-yl)-pyrazolo[1,5-*c*]quinazoline-1,2-dicarboxylate (5). To a suspension of **38** (1.1 mmol) in anhydrous pyridine (6 mL), diformylhydrazine (3.3 mmol) and, drop by drop, trimethylsilylchloride (16.5 mmol) and triethylamine (7.7 mmol) were successively added. The resulting solution was refluxed for 4 h, then cooled at room temperature, and diluted with water (30 mL). The resulting solid was collected and washed with water. ^{1}H NMR 1.21 (t, 3H, al, J = 7.2 Hz), 1.32 (t, 3H, al, J = 7.3 Hz), 4.28–4.39 (m, 4H, al), 7.61 (s, 1H, ar), 8.90–8.93 (m, 3H, ar), 12.70 (br s, 1H, NH). IR 1722, 1738, 3105.

5.1.6. Synthesis of diethyl 8-chloro-5,6-dihydro-5-oxo-9-(pyrrol-1-yl)-pyrazolo[1,5-*c*]quinazoline-1,2-dicarboxylate (6). To a hot (90 °C) suspension of **38** (0.8 mmol) in glacial acetic acid (10 mL), a solution of 2,5-diethoxytetrahydrofuran (1.18 mmol) in glacial acetic acid (5 mL) was added dropwise.

The mixture was heated at 90 °C for 15 min and then cooled and diluted with water (20 mL). The solid was collected and washed with water. ^{1}H NMR 1.24 (t, 3H, al, J = 7.2 Hz), 1.34 (t, 3H, al, J = 7.2 Hz), 4.31

(q, 2H, al, J = 7.2 Hz), 4.40 (q, 2H, al, J = 7.2 Hz), 6.30–6.31 (m, 2H, ar), 7.02–7.03 (m, 2H, ar), 7.58 (s, 1H, ar), 8.86 (s, 1H, ar), 12.61 (br s, 1H, NH). IR 1727, 1747, 3564.

5.1.7. Synthesis of diethyl 8-chloro-5,6-dihydro-9-(3-formylpyrrol-1-yl)-5-oxo-pyrazolo[1,5-*c*]quinazoline-1,2-dicarboxylate (7). To a hot (90 °C) suspension of **38** (0.58 mmol) in glacial acetic acid (4 mL), a solution of 2,5-dimethoxy-3-tetrahydrofurancarboxaldehyde (0.87 mmol) in glacial acetic acid (3 mL) was added dropwise. The mixture was heated at 90 °C for 20 min and then evaporation at reduced pressure of the solvent yielded a residue that was collected and purified by column chromatography (eluting system acetone/chloroform/cyclohexane 6/4/0.2). Evaporation of the first eluates afforded the desired compound. ^{1}H NMR 1.23 (t, 3H, al, J = 7.1 Hz), 1.34 (t, 3H, al, J = 7.1 Hz), 4.31 (q, 2H, al, J = 7.1 Hz), 4.40 (q, 2H, al, J = 7.1 Hz), 6.72 (s, 1H, ar), 7.21 (s, 1H, ar), 7.61 (s, 1H, ar), 7.97 (s, 1H, ar), 8.97 (s, 1H, ar), 9.81 (s, 1H, CHO), 12.70 (br s, 1H, NH). IR 1731, 1751, 3113.

5.1.8. Synthesis of 1,8-dichloro-5,6-dihydro-9-nitro-5-oxo-pyrazolo[1,5-*c*]quinazoline-2-carboxylic acid (8)[21]. To a suspension of the ester **2** (0.54 mmol) in methanol (4 mL), an aqueous solution of KOH (10% w/w, 3.7 mL) was added and then the mixture was refluxed for 10 min. After cooling at room temperature, addition of water (20 mL) and acidification with 6 N HCl afforded a solid residue that was collected by filtration and washed with water. ^{1}H NMR 7.51 (s, 1H, ar), 9.07 (s, 1H, ar), 13.20 (br s, 1H, exchangeable proton).

5.1.9. Synthesis of 8-chloro-5,6-dihydro-1,9-dinitro-5-oxo-pyrazolo[1,5-*c*]quinazoline-2-carboxylic acid (9).[21] The title compound was prepared from **3** (0.40 mmol) following the experimental procedure described above for the preparation of **8**. ^{1}H NMR 7.58 (s, 1H, ar), 9.24 (s, 1H, ar). IR 1750, 1950, 3200, 3450.

5.1.10. Synthesis of 8-chloro-1-ethoxycarbonyl-5,6-dihydro-9-nitro-5-oxo-pyrazolo[1,5-*c*]quinazoline-2-carboxylic acid (10).[21] To a suspension of **4** (0.74 mmol) in methanol (6 mL), an aqueous solution of KOH (10% w/w, 6 mL) was added. The mixture was left at room temperature under stirring for 1 h. Addition of water (15 mL) and acidification with 12 N HCl afforded a suspension that was kept at room temperature for 1 h. The solid was collected and washed with water. ^{1}H NMR 1.30 (t, 3H, al, J = 6.9 Hz), 4.35 (q, 2H, al, J = 6.9 Hz), 7.52 (s, 1H, ar), 9.51 (s, 1H, ar), 12.84 (br s, 1H, exchangeable proton). IR 1697, 1743, 3206, 3390, 3497.

5.1.11. Synthesis of 8-chloro-5,6-dihydro-9-nitro-5-oxo-pyrazolo[1,5-*c*]quinazoline-1,2-dicarboxylic acid (11).[21] A solution of KOH (10% w/w, 7 mL) was added to a suspension of **4** (0.6 mmol) in ethanol (7 mL). The mixture was heated at 100 °C for 1 h and then diluted with water (15 mL). Acidification with 12 N HCl yielded a suspension that, after heating at 100 °C for 30 min and stirring at room temperature for 12 h, afforded a solid which was collected by filtration and washed with water. ^{1}H NMR 7.52 (s, 1H, ar), 9.86 (s, 1H, ar), 12.79 (br s, 1H, exchangeable proton). IR 1753, 3171, 3431, 3494.

5.1.12. Synthesis of 8-chloro-5,6-dihydro-5-oxo-9-(1,2,4-triazol-4-yl)-pyrazolo[1,5-*c*]quinazoline-1,2-dicarboxylic acid (12). The title compound was obtained from **5** following Section 5 described above for the preparation of **11.** ^{1}H NMR 7.59 (s, 1H, ar), 8.93 (s, 2H ar), 9.22 (s, 1H, ar), 12.63 (br s, 1H, exchangeable proton). IR 1712, 1737, 3257, 3444.

5.1.13. Synthesis of 8-chloro-5,6-dihydro-5-oxo-9-(pyrrol-1-yl)-pyrazolo[1,5-*c*]quinazoline-1,2-dicarboxylic acid (13). The ester **6** (8.4 mmol) was dissolved at room temperature in 15 mL of 1 M NaOH. The mixture was stirred at room temperature for 12 h, and then diluted with water (20 mL). Acidification in an ice bath with 6 N HCl afforded an unfilterable suspension that after stirring at room temperature for 12 h yielded a solid which was collected and washed with water. ^{1}H NMR 6.29 (m, 2H, pyrrole protons), 7.01 (m, 2H, pyrrole protons), 7.56 (s, 1H, ar), 9.05 (s, 1H, ar), 12.47 (br s 1H, exchangeable proton). IR 1720, 1741, 3248, 3456.

5.1.14. General procedure to prepare diethyl 8-chloro-9-[3-substituted-ureido]-5,6-dihydro-5-oxo-pyrazolo[1,5-*c*]quinazoline-1,2-dicarboxylates (14–23). To a hot (70 °C) solution of **38** (0.80 mmol) in anhydrous tetrahydrofuran (25 mL), an excess of the appropriate isocyanate (1.1 mmol) was added. The reaction mixture was stirred at 70 °C and under nitrogen atmosphere for 3 days. Once a day, an equimolar amount of the suitable isocyanate was added. When the reaction was complete (TLC monitoring: eluting system $CHCl_3$/MeOH 9:1), the mixture was cooled at room temperature. Compounds **14**, **17**–**22** precipitated and were then collected by filtration and recrystallized. Compounds **15**–**16** and **23** were soluble and thus evaporation at reduced pressure of the solvent yielded a residue which was purified by column chromatography (compound **15** eluting system chloroform/ethanol 9.5:0.5) or by crystallization (compounds **16** and **23**).

5.1.14.1. Diethyl 8-chloro-9-[3-(ethoxycarbonylmethyl)ureido]-5,6-dihydro-5-oxo-pyrazolo[1,5-*c*]quinazoline-1,2-dicarboxylate (14). ^{1}H NMR 1.20–1.35 (m, 9H, al), 3.92–3.93 (m, 2H, al), 4.13 (q, 2H, al, J = 7.0 Hz), 4.37–4.43 (m, 4H, al), 7.29–7.32 (m, 1H, NH), 7.42 (s, 1H, ar), 8.48 (s, 1H, NH), 8.99 (s, 1H, ar), 12.26 (br s, 1H, NH). IR 1693, 1727, 1740, 3352.

5.1.14.2. Diethyl 8-chloro-9-[3-(2-ethoxycarbonylethyl)ureido]-5,6-dihydro-5-oxo-pyrazolo[1,5-*c*]quinazoline-1,2-dicarboxylate (15). ^{1}H NMR (pyridine-d_5) 1.05 (t, 3H, al, J = 7.1 Hz), 1.23 (t, 3H, al, J = 7.0 Hz), 1.47 (t, 3H, al, J = 6.9 Hz), 2.69–2.74 (m, 2H, al), 3.79–3.81 (m, 2H, al), 3.99–4.03 (m, 2H, al), 4.40–4.45 (m, 2H, al), 4.79–4.83 (m, 2H, al,), 7.43 (s, 1H, ar), 7.84–7.89 (m, 1H, NH), 8.54 (s, 1H, NH), 9.77 (s, 1H, ar).

5.1.14.3. Diethyl 8-chloro-9-[3-(3-ethoxycarbonylpropyl)ureido]-5,6-dihydro-5-oxo-pyrazolo[1,5-*c*]quinazoline-1,2-dicarboxylate (16). ^{1}H NMR 1.18 (t, 3H, al, J = 7.1 Hz), 1.30–1.35 (m, 6H, al), 1.69–1.74 (m, 2H,

al), 2.35 (t, 2H, al, J = 7.5 Hz), 3.11–3.16 (m, 2H, al), 4.06 (q, 2H, al, J = 7.1 Hz), 4.37 (q, 2H, al, J = 7.1 Hz), 4.43 (q, 2H, al, J = 7.1 Hz), 6.99–7.02 (m, 1H, NH), 7.40 (s, 1H, ar), 8.14 (s, 1H, NH), 9.01 (s, 1H, ar), 12.26 (br s, 1H, NH). IR 1731, 3319.

5.1.14.4. Diethyl 8-chloro-9-(3-ethylureido)-5,6-dihydro-5-oxo-pyrazolo[1,5-*c*]quinazoline-1,2-dicarboxylate (17). ^{1}H NMR 1.07 (t, 3H, al, J = 5.5 Hz), 1.31–1.36 (m, 6H, al), 3.13–3.16 (m, 2H, al), 4.38 (q, 2H, al, J = 7.0 Hz), 4.45 (q, 2H, al, J = 7.0 Hz), 6.92–6.94 (m, 1H, NH), 7.41 (s, 1H, ar), 8.12 (s, 1H, NH), 9.02 (s, 1H, ar), 12.23 (br s, 1H, NH). IR 1731, 3127, 3328.

5.1.14.5. Diethyl 8-chloro-9-(3-phenylureido)-5,6-dihydro-5-oxo-pyrazolo[1,5-*c*]quinazoline-1,2-dicarboxylate (18). ^{1}H NMR 1.32–1.37 (m, 6H, al), 4.39 (q, 2H, al, J = 6.9 Hz), 4.47 (q, 2H, al, J = 6.9 Hz), 6.99–7.03 (m, 1H, ar), 7.30–7.34 (m, 2H, ar), 7.46–7.50 (m, 3H, ar), 8.47 (s, 1H, NH), 9.08 (s, 1H, ar), 9.39 (s, 1H, NH), 12.31 (s, 1H, NH).

5.1.14.6. Diethyl 8-chloro-9-(3-benzylureido)-5,6-dihydro-5-oxo-pyrazolo[1,5-*c*]quinazoline-1,2-dicarboxylate (19). ^{1}H NMR 1.29–1.35 (m, 6H, al), 4.33–4.44 (m, 6H, al), 7.27–7.42 (m, 7H, 6ar + NH), 8.27 (s, 1H, NH), 9.04 (s, 1H, ar), 12.25 (br s, 1H, NH).

5.1.14.7. Diethyl 8-chloro-9-[3-(2-ethylphenyl)ureido]-5,6-dihydro-5-oxo-pyrazolo[1,5-*c*]quinazoline-1,2-dicarboxylate (20). ^{1}H NMR 1.32–1.36 (m, 6H, al), 2.77–2.80 (m, 2H, al), 3.36–3.39 (m, 2H, al), 4.38 (q, 2H, al, J = 6.9 Hz), 4.46 (q, 2H, al, J = 7.0 Hz), 6.95–6.98 (m, 1H, NH), 7.21–7.35 (m, 5H, ar), 7.44 (s, 1H, ar), 8.18 (s, 1H, NH), 9.02 (s, 1H, ar), 12.21 (br s, 1H, NH).

5.1.14.8. Diethyl 8-chloro-9-[3-(3-ethoxycarbonylphenyl)ureido]-5,6-dihydro-5-oxo-pyrazolo[1,5-*c*]quinazoline-1,2-dicarboxylate (21). ^{1}H NMR 1.34–1.39 (m, 9H, al), 4.33 (q, 2H, al, J = 7.3 Hz), 4.36 (q, 2H, al, J = 7.3 Hz), 4.50 (q, 2H, al, J = 7.0 Hz), 7.46–7.49 (m, 2H, ar), 7.60–7.62 (m, 2H, ar), 8.25 (s, 1H, ar), 8.50 (s, 1H, NH), 9.09 (s, 1H, ar), 9.66 (s, 1H, NH), 12.33 (br s, 1H, NH).

5.1.14.9. Diethyl 8-chloro-9-[3-(4-ethoxycarbonylphenyl)ureido]-5,6-dihydro-5-oxo-pyrazolo[1,5-*c*]quinazoline-1,2-dicarboxylate (22). ^{1}H NMR 1.29–1.37 (m, 9H, al), 4.28 (q, 2H, al, J = 7.1 Hz), 4.38 (q, 2H, al, J = 7.1 Hz), 4.46 (q, 2H, al, J = 7.1 Hz), 7.46 (s, 1H, ar), 7.62 (d, 2H, ar, J = 8.6 Hz), 7.93 (d, 2H, ar, J = 8.6 Hz), 8.60 (s, 1H, NH), 9.07 (s, 1H, ar), 9.78 (s, 1H, NH), 12.34 (br s, 1H, NH).

5.1.14.10. Diethyl (±)8-chloro-9-[3-(1-methoxycarbonyl-1-benzyl-methyl)ureido]-5,6-dihydro-5-oxo-pyrazolo-[1,5-*c*]quinazoline-1,2-dicarboxylate (23). ^{1}H NMR 1.28–1.34 (m, 6H, al), 2.96–3.01 (m, 1H, al), 3.06–3.11 (m, 1H, al), 3.65 (s, 3H, al), 4.35–4.43 (m, 4H, al), 4.52–4.57 (m, 1H, al), 7.21–7.41 (m, 7H, 6ar + NH), 8.44 (s, 1H, NH), 8.96 (s, 1H ar), 12.25 (br s, 1H, NH). IR 1732, 3287, 3368.

5.1.15. General procedure to prepare 8-chloro-9-[3-substituted-ureido]-5,6-dihydro-5-oxo-pyrazolo[1,5-*c*]quinazoline-1,2-dicarboxylic acids (24–32). To a suspension of the esters **14–15**, **17–23** (0.84 mmol) in a mixture of water and ethanol (1:1; 10 mL), a 10% w/w NaOH solution (5 mL) was added. The reaction mixture was refluxed until disappearance of the starting material (TLC monitoring: eluting system $CHCl_3$/MeOH/AcOH 7:1:2). The solution was diluted with water (15 mL) and then acidified with 6 N HCl. The suspension was refluxed for 10 min and then cooled to room temperature. The solid was collected and washed with water.

5.1.15.1. 8-Chloro-9-[3-(carboxymethyl)ureido]-5,6-dihydro-5-oxo-pyrazolo[1,5-*c*]quinazoline-1,2-dicarboxylic acid (24). ^{1}H NMR 3.85 (d, 2H, al, J = 5.0 Hz), 7.20 (br s, 1H, NH), 7.41 (s, 1H, ar), 8.42 (s, 1H, NH), 9.25 (s, 1H, ar), 12.19 (s, 1H, exchangeable proton).

5.1.15.2. 8-Chloro-9-[3-(2-carboxyethyl)ureido]-5,6-dihydro-5-oxo-pyrazolo[1,5-*c*]quinazoline-1,2-dicarboxylic acid (25). ^{1}H NMR 7.02 (br s, 1H, NH), 7.39 (s, 1H, ar), 8.25 (s, 1H, NH), 9.27 (s, 1H, ar), 12.17 (s, 1H, exchangeable proton). ^{1}H NMR (CD_3OD-d_4) 2.59–2.60 (m, 2H, al), 3.51–3.52 (m, 2H, al), 7.43 (s, 1H, ar), 9.39 (s, 1H, ar). IR 1722, 3360.

5.1.15.3. 8-Chloro-9-(3-ethylureido)-5,6-dihydro-5-oxo-pyrazolo[1,5-*c*]quinazoline-1,2-dicarboxylic acid (26). ^{1}H NMR 1.07 (t, 3H, al, J = 7.2 Hz), 3.10–3.16 (m, 2H, al), 6.82 (br s, 1H, NH), 7.38 (s, 1H, ar), 8.08 (s, 1H, NH), 9.28 (s, 1H, ar), 12.18 (s, 1H, exchangeable proton). IR 1710, 1743, 3328.

5.1.15.4. 8-Chloro-9-(3-phenylureido)-5,6-dihydro-5-oxo-pyrazolo[1,5-*c*]quinazoline-1,2-dicarboxylic acid (27). ^{1}H NMR 6.98–7.02 (m, 1H, ar), 7.29–7.33 (m, 2H, ar), 7.45–7.49 (m, 3H, ar), 8.39 (s, 1H, NH), 9.26 (s, 1H, ar), 9.48 (s, 1H, NH), 12.21 (s, 1H, exchangeable proton).

5.1.15.5. 8-Chloro-9-(3-benzylureido)-5,6-dihydro-5-oxo-pyrazolo[1,5-*c*]quinazoline-1,2-dicarboxylic acid (28). ^{1}H NMR 4.35 (d, 2H, al, J = 5.5 Hz), 7.27–7.41 (m, 7H, 6ar + NH), 8.22 (s, 1H, NH), 9.33 (s, 1H, ar), 12.17 (s, 1H, exchangeable proton).

5.1.15.6. 8-Chloro-9-[3-(2-phenylethyl)ureido]-5,6-dihydro-5-oxo-pyrazolo[1,5-*c*]quinazoline-1,2-dicarboxylic acid (29). ^{1}H NMR 2.76–2.79 (m, 2H, al), 3.36–3.39 (m, 2H, al), 6.86–6.87 (m, 1H, NH), 7.22–7.33 (m, 5H, ar), 7.44 (s, 1H, ar), 8.16 (s, 1H, NH), 9.23 (s, 1H, ar).

5.1.15.7. 8-Chloro-9-[3-(3-carboxyphenyl)ureido]-5,6-dihydro-5-oxo-pyrazolo[1,5-*c*]quinazoline-1,2-dicarboxylic acid (30). ^{1}H NMR 7.40–7.43 (m, 2H, ar), 7.56 (d, 1H, ar, J = 7.6 Hz), 7.67 (d, 1H, ar, J = 7.8 Hz), 8.14 (s, 1H, ar), 8.40 (s, 1H, NH), 9.38 (s, 1H, ar), 9.89 (br s, 1H, NH), 12.21 (s, 1H, exchangeable proton) 12.96 (br s, 1H, exchangeable proton). IR 1693, 1746, 3284.

5.1.15.8. 8-Chloro-9-[3-(4-carboxyphenyl)ureido]-5,6-dihydro-5-oxo-pyrazolo[1,5-*c*]quinazoline-1,2-dicarboxylic acid (31). ^{1}H NMR 7.46 (s, 1H, ar), 7.59 (d, 2H, ar,

J = 8.7 Hz), 7.90 (d, 2H, ar, J = 8.7 Hz), 8.55 (s, 1H, NH), 9.37 (s, 1H, ar), 9.68 (s, 1H, NH), 12.27 (br s, 1H, exchangeable proton). IR 1723, 3359, 3611.

5.1.15.9. (±) 8-Chloro-9-[3-(1-benzyl-1-carboxymethyl) ureido]-5,6-dihydro-5-oxo-pyrazolo[1,5-*c*]quinazoline-1,2-dicarboxylic acid (32). ^{1}H NMR 2.93–2.98 (m, 1H, al), 3.09–3.14 (m, 1H, al), 4.47–4.48 (m, 1H, al), 7.20–7.34 (m, 6H, 5ar + NH), 7.40 (s, 1H, ar), 8.39 (s, 1H, NH), 9.28 (s, 1H ar), 12.18 (br s, 1H, exchangeable proton).

5.1.16. Synthesis of diethyl 8-chloro-9-[(2-carboxybenzoyl)amino]-5,6-dihydro-5-oxo-pyrazolo[1,5-*c*]quinazoline-1,2-dicarboxylate (33). To a hot (60 °C) suspension of **38** (0.90 mmol) in glacial acetic acid (5 mL), sodium acetate (0.90 mmol) and phthalic anhydride (1.18 mmol) were added. The reaction mixture was heated at 60 °C for 10 min, then addition of water afforded a solid which was collected and washed with water. ^{1}H NMR 1.31–1.36 (m, 6H, al), 4.37–4.45 (m, 4H, al), 7.50 (s, 1H, ar), 7.59–7.61 (m, 2H, ar), 7.67–7.70 (m, 1H, ar), 7.88–7.90 (m, 1H, ar), 8.77 (s, 1H, ar), 10.23 (br s, 1H, exchangeable proton), 12.43 (s, 1H, exchangeable proton). IR 1715, 1756, 3160, 3461.

5.1.17. Synthesis of 8-chloro-9-[(2-carboxybenzoyl) amino]-5,6-dihydro-5-oxo-pyrazolo[1,5-*c*]quinazoline-1,2-dicarboxylic acid (34). The title compound was obtained by alkaline hydrolysis of ester **33** (0.46 mmol) following Section 5 described above for the synthesis of **13**. ^{1}H NMR 7.50 (s, 1H, ar), 7.60–7.68 (m, 3H, ar), 7.85–7.87 (m, 1H, ar), 9.12 (s, 1H, ar), 10.22 (br s, 1H, NH), 12.33 (br s, 1H, exchangeable proton).

6. Biochemistry

6.1. Binding assay

Rat cortical synaptic membrane preparation and [^{3}H]glycine, [^{3}H]AMPA, and high-affinity [^{3}H]kainate binding experiments were performed following the procedures described in Refs. 16, 22, and 23, respectively.

6.2. Electrophysiological assay

The cortical wedge preparation described by Mannaioni et al.[24] was used while the electrophysiological assays were performed following the procedures described in Ref. 16.

6.3. Sample preparation and results calculation

A stock 1 mM solution of the tested compound was prepared in 50% DMSO. 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 test compound using the ALLFIT computer program[25] and, in the case of tritiated glycine and AMPA binding, converted to K_i values by application of the Cheng–Prusoff equation.[26] Under our experimental conditions, the dissociation constants (K_D) for [^{3}H]glycine (10 nM) and [^{3}H]-DL-AMPA (8 nM) were 75 ± 6 and 28 ± 3 nM, respectively.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2007.11.046.

References and notes

1. Bräuner-Osborne, H.; Egebjerg, J.; Nielsen, E. Ø.; Madsen, U.; Krogsgaard-Larsen, P. *J. Med. Chem.* **2000**, *43*, 2609.
2. Dingledine, R.; Borges, K.; Bowie, D.; Traynelis, S. F. *Pharmacol. Rev.* **1999**, *51*, 7.
3. Johnson, J. W.; Ascher, P. *Nature* **1987**, *325*, 529.
4. Wahlgren, N. G. *Int. Rev. Neurobiol.* **1997**, *40*, 337.
5. Hallett, P. J.; Standaert, D. G. *Pharmacol. Ther.* **2004**, *102*, 155.
6. Hynd, M. R.; Scott, H. L.; Dodd, P. R. *Neurochem. Int.* **2004**, *45*, 583.
7. Pitt, D.; Werner, P.; Raine, C. S. *Nature* **2000**, *6*, 67.
8. Catarzi, D.; Colotta, V.; Varano, F. *Med. Res. Rev.* **2007**, *27*, 239.
9. Catarzi, D.; Colotta, V.; Varano, F. *Curr. Top. Med. Chem.* **2006**, *6*, 809.
10. Bleakman, D.; Lodge, D. *Neuropharmacology* **1998**, *37*, 1187.
11. Lerma, J.; Paternain, A. V.; Rodriguez-Moreno, A.; Lòpez-Garcìa, J. C. *Physiol. Rev.* **2001**, *81*, 971.
12. Bleakman, D.; Gates, M. R.; Ogden, A. M.; Mackowiak, M. *Curr. Pharm. Des.* **2002**, *8*, 873.
13. Shou, X.; Chamberlin, A. R. *Exp. Opin. Ther. Patents* **2004**, *14*, 471.
14. Dolman, N. P.; Troop, H. M.; More, J. C. A.; Alt, A.; Knaus, J. L.; Nistico, R.; Jack, S.; Morley, R. M.; Bortolotto, Z. A.; Roberts, P. J.; Bleackman, D.; Collingridge, G. L.; Jane, D. E. *J. Med. Chem.* **2005**, *48*, 7867.
15. Colotta, V.; Catarzi, D.; Varano, F.; Lenzi, O.; Filacchioni, G.; Costagli, C.; Galli, A.; Ghelardini, C.; Galeotti, N.; Gratteri, P.; Sgrignani, J.; Deflorian, F.; Moro, S. *J. Med. Chem.* **2006**, *49*, 6015.
16. Varano, F.; Catarzi, D.; Colotta, V.; Filacchioni, G.; Galli, A.; Costagli, C.; Carlà, V. *J. Med. Chem.* **2002**, *45*, 1035.
17. Varano, F.; Catarzi, D.; Colotta, V.; Calabri, F. R.; Lenzi, O.; Filacchioni, G.; Galli, A.; Costagli, C.; Deflorian, F.; Moro, S. *Bioorg. Med. Chem.* **2005**, *13*, 5536.
18. Catarzi, D.; Colotta, V.; Varano, F.; Cecchi, L.; Filacchioni, G.; Galli, A.; Costagli, C.; Carlà, V. *J. Med. Chem.* **2000**, *43*, 3824.
19. Catarzi, D.; Colotta, V.; Varano, F.; Filacchioni, G.; Galli, A.; Costagli, C.; Carlà, V. *J. Med. Chem.* **2001**, *44*, 3157.
20. Catarzi, D.; Colotta, V.; Varano, F.; Calabri, F. R.; Filacchioni, G.; Galli, A.; Costagli, C.; Carlà, V. *J. Med. Chem.* **2004**, *47*, 262.
21. Moro, S.; Varano, F.; Cozza, G.; Pagano, M.; Fagotto, G.; Chilin, A.; Guiotto, A.; Catarzi, D.; Colotta, V.; Pinna, L. A.; Meggio, F. *Lett. Drug Des. Discov.* **2006**, *3*, 281.
22. Colotta, V.; Catarzi, D.; Varano, F.; Cecchi, L.; Filacchioni, G.; Galli, A.; Costagli, C. *Arch. Pharm. Pharm. Med. Chem.* **1997**, *330*, 129.
23. Nielsen, E. O.; Madsen, U.; Schaumburg, K.; Brehm, L.; Krogsgaard-Larsen, P. *Eur. J. Chem. Chim. Ther.* **1986**, *21*, 433.
24. Mannaioni, G.; Carlà, V.; Moroni, F. *Br. J. Pharmacol.* **1996**, *118*, 1530.
25. De Lean, A.; Munson, P. J.; Rodbard, D. *Am. J. Physiol.* **1978**, *235*, E97.
26. Cheng, Y.-C.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099.