Synthesis and Biological Evaluation of a New Set of Pyrazolo[1,5-*c*]quinazolines as Glycine/*N*-Methyl-D-aspartic Acid Receptor Antagonists

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Previous studies have shown that 8-chloro-5,6-dihydro-5-oxo-pyrazolo[1,5-*c*]quinazoline-2-carboxylates (PQZ series) represent a family of glycine/*N*-methyl-D-aspartic acid (NMDA) and/or (*R*,*S*)-2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid (AMPA) and/or kainic acid (KA) receptor antagonists. Moreover, some groups have been identified that introduced in suitable positions of the PQZ 2-carboxylate framework shift affinity and selectivity toward glycine/NMDA receptor. These substituents are a carboxylate function at position-1 and/or a chlorine atom at position-9. In this paper we report a study on some new 5,6-dihydro-5-oxo-pyrazolo[1,5-*c*]quinazoline-1-carboxylates bearing at position-2 a lipophilic amide group or lacking substituent at this same position. All the newly synthesised compounds were evaluated for their binding at glycine/NMDA, AMPA and KA receptors. These studies led to the identification of some new PQZ derivatives endowed with good glycine/NMDA receptor affinity and selectivity and to better definition of the structure–activity relationship (SAR) of this class of compounds.

Key words ionotropic glutamate receptor; glycine and *N*-methyl-D-aspartic acid receptor; pyrazoloquinazoline; *N*-methyl-D-aspartic acid

The amino acid neurotransmitter L-glutamate (Glu) plays a pivotal role in the excitatory pathways of the mammalian central nervous system where it is involved in the physiological regulation of processes such as neurotransmission, synaptic plasticity, learning and memory.[1—3)]

Glu mediates its effects *via* activation of metabotropic (mGluRs) and ionotropic (iGluRs) receptors, the latter consisting of two major subclasses: *N*-methyl-D-aspartic acid (NMDA) and non-NMDA receptors (kainic acid (KA) and (*R*,*S*)-2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid (AMPA) receptors).[4)]

It is well known that excessive release of Glu from presynaptic terminals and the subsequent overstimulation of postsynaptic GluRs cause excitotoxic mechanisms that play a critical role in the pathophysiology of several acute and chronic neurological pathologies such as cerebral ischemia, epilepsy, Alzheimer's and Parkinson's diseases, and amyotrophic lateral sclerosis.[2,5)]

Antagonists of NMDA or non-NMDA receptors are therefore of great therapeutic interest for the treatment of brain diseases which are pathophysiologically linked to excessive excitatory amino acid receptor activation.[2)]

Targeting the NMDA receptor is a promising approach to anti-excitotoxic therapy, however competitive NMDA receptor antagonists cause undesirable side effects such as hallucinations, ataxia, and motor incoordination that prevent their clinical use. Aiming at the glycine coagonist site of the NMDA receptor complex may bypass these shortcomings. In addition, glycine/NMDA receptor antagonists have other potential therapeutic applications, such as for the treatment of traumatic brain injury, chronic pain, drug and alcohol abuse and tolerance.[6—15)]

Over the past decade, our laboratory has been notably involved in the elucidation of the structure–activity relationship (SAR) of different chemical classes of heterocyclic compounds acting as glycine/NMDA and/or AMPA and/or KA receptor antagonists.[16—29)] One of these classes is represented by the 8-chloro-5,6-dihydro-5-oxo-pyrazolo[1,5-*c*]quinazoline-2-carboxylates (PQZ series) (Fig. 1) bearing various substituents on the fused benzo ring and at position-1 (compounds **A**—**D**).[21,25,28)] These studies have provided evidence of the structural requirements which are important to obtain iGluR receptor antagonists: i) a NH proton donor that binds to a proton acceptor of the receptor site; ii) the 3 nitrogen atom and the oxygen atom of the 5-carbonyl group that are δ-negatively charged heteroatoms able to form a coulombic interaction with a positive site of the receptor; iii) a carboxylate function at position-2 able to engage a strong hydrogen-bond interaction with a cationic proton donor site of the receptor; and iv) an electron-withdrawing substituent at position-8 on the fused benzo moiety. These studies have also shown that introduction of a carboxylate moiety at position-1 or of a chlorine atom at position-9 of the parent compound **A** shifts affinity and selectivity toward glycine/NMDA receptor (compounds **B** and **C**, respectively) (Fig. 1). Finally it has been demonstrated that the contemporary presence of these substituents (compound **D**) is well tolerated by the glycine/NMDA receptor (Fig. 1).

To gain more information from SAR studies and in the attempt to optimize glycine/NMDA receptor affinity and selectivity of PQZ derivatives, a new set of related analogues **1**—

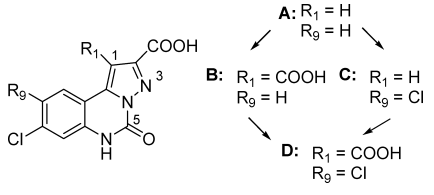

Fig. 1. Previously Reported PQZ Derivatives

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10 was developed (Fig. 2). Compounds **1**—**10**, while maintaining a carboxylate function at position-1, possess an amide moiety at position-2 (compounds **1**—**6**) or lack a substituent at this same position (compounds **7**—**10**) (Fig. 2). It has to be noted that **1**—**6** bear a lipophilic amide side chain similarly to a number of bicyclic or tricyclic heteroaromatic derivatives reported as potent and selective glycine/NMDA receptor antagonists.[15] Moreover, the glycine/NMDA receptor pharmacophore model reported in the literature[15,30] shows a hydrophobic pocket that well accommodates bulky groups in a region of the receptor site corresponding to position-2 of the PQZ scaffold.

All the newly synthesised compounds were biologically evaluated for their binding at the glycine/NMDA receptor. AMPA and KA high-affinity binding assays were also performed to assess the selectivity of the reported compounds toward the glycine/NMDA receptor.

Chemistry The target derivatives **1**—**10** were prepared as depicted in Chart 1. The starting 1-(ethoxycarbonyl)-5,6-dihydro-5-oxo-pyrazolo[1,5-*c*]quinazoline-2-carboxylic acids **11**, **12**, and the ethyl 5,6-dihydro-5-oxo-pyrazolo[1,5-*c*]quinazoline-1-carboxylates **7** and **8** were prepared as previously reported.[25] Reaction of **11** and **12** with suitable amines gave the 2-carboxyamides **1**—**3**. Finally, by treatment of esters **1**—**3**, **7**, **8** in alkaline medium the corresponding acids **4**—**6**, **9**, **10** were obtained.

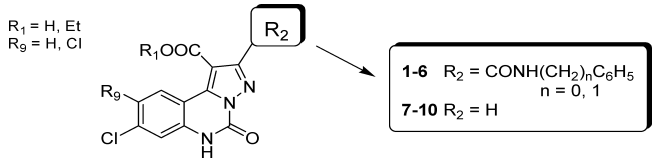
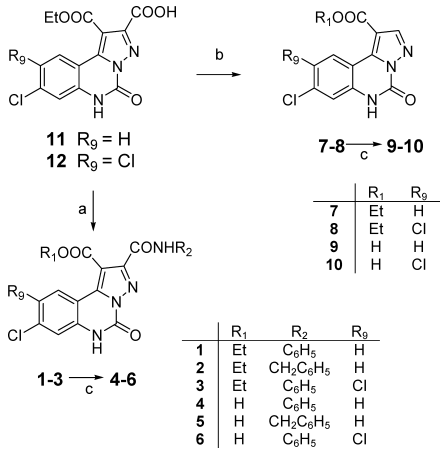


Fig. 2. Currently Reported PQZ Derivatives

Results and Discussion

The pyrazoloquinazolines **1**—**10**, together with **DCKA** (5,7-dichlorokynurenic acid) and **NBQX** (2,3-dihydroxy-6-nitro-7-sulphamoylbenzo[*f*]-quinoxaline) as standard compounds, were tested for their ability to displace tritiated glycine from its specific binding in rat cortical membranes. AMPA and high-affinity KA binding assays were also performed on rat cortical membranes. The binding results are shown in Table 1 together with those of the previously reported PQZ derivatives **B**[25] **C**[21] and **D**[25] included as reference compounds.

The binding results indicate that synthesis of compounds **1**—**10** produced some new glycine/NMDA receptor antagonists. Indeed, the 2-amido **4**—**6** and the 2-unsubstituted derivatives **8** and **10** are endowed with glycine/NMDA receptor



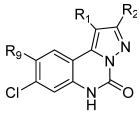
	R1	R9
7	Et	H
8	Et	Cl
9	H	H
10	H	Cl

	R1	R2	R9
1	Et	C_6H_5	H
2	Et	$CH_2C_6H_5$	H
3	Et	C_6H_5	Cl
4	H	C_6H_5	H
5	H	$CH_2C_6H_5$	H
6	H	C_6H_5	Cl

Reagents: (a) EDC hydrochloride, HOBt, R_2NH_2, DMF, room temperature; (b) heating over the melting points; (c) i) 2 M KOH/EtOH, room temperature, ii) 6 M HCl.

Chart 1

Table 1. Binding Affinities at Glycine/NMDA, AMPA and KA Receptors



B, C, D, 1-10

Compd.	R_9	R_1	R_2	K_i (μM)[a] or I%[b]		Selectivity ratio[c]	IC_{50} (μM)[d] or I%[b]
				[^{3}H]glycine	[^{3}H]AMPA		[^{3}H]kainate
B[e]	H	COOH	COOH	0.09±0.01	1.1±0.2	12.2	11.5±1.4
C[f]	Cl	H	COOH	0.16±0.04	2.4±0.8	15	41±3.0
D[e]	Cl	COOH	COOH	0.059±0.01	0.71±0.08	12	19.5±1.1
1	H	COOEt	CONHPh	9.5±2.0	42%	>10	19%
2	H	COOEt	$CONHCH_2Ph$	44±6.0	18±0.9	0.4	7%
3	Cl	COOEt	CONHPh	28%	16%	—	9%
4	H	COOH	CONHPh	0.58±0.08	3.4±0.3	5.9	18.5±1.2
5	H	COOH	$CONHCH_2Ph$	0.52±0.05	3.8±0.2	7.3	33±2
6	Cl	COOH	CONHPh	0.12±0.01	1.8±0.3	15	17±3
7	H	COOEt	H	36%	32%	—	4%
8	Cl	COOEt	H	0.70±0.15	8.3±2.5	11.8	91±11
9	H	COOH	H	2.5±0.4	6.4±0.7	2.6	48%
10	Cl	COOH	H	0.27±0.05	5.8±0.8	21.5	27.5±3.4
DCKA	—	—	—	0.09±0.02	5%	>1000	8%
NBQX	—	—	—	3%	0.07±0.06	<1000	7.0±1.1

a) Inhibition constant (K_i) values were means±S.E.M. of three or four separate determinations in triplicate. *b*) Percentage of inhibition (I%) of specific binding at 100 μM concentration. *c*) Glycine/NMDA *versus* AMPA selectivity ratio. *d*) Concentrations necessary for 50% inhibition (IC_{50}). The IC_{50} values were means±S.E.M. of three or four separate determinations in triplicate. *e*) Ref. 25. *f*) Ref. 21.

affinities in the low micromolar range. Affinities of compounds **1**—**10** for both AMPA and KA receptors are generally lower than those for the glycine/NMDA one even if they generally show AMPA receptor binding affinities in the micromolar range.

Replacement of the 2-carboxylic group of lead compounds **B** and **D** with a lipophylic amide side chain is tolerated by all three receptor types. Generally, **4**—**6** bind to the receptor sites even if with lower affinities with respect to those of **B** and **D**. However, it has to be noted that glycine/NMDA and AMPA receptor affinities of the 2-phenylcarbamoyl-1-carboxylic acid **6** are only about 2-fold lower than those of the previously reported 1,2-dicarboxylic acid **B**. As expected **1**, **2**, **4**—**6** show higher glycine/NMDA receptor binding affinities than those at AMPA and KA receptors, thus confirming that the presence of a lipophilic side chain is tolerated especially by the glycine/NMDA receptor.

Particularly interesting are the binding data, in general in the micromolar range, of the 1-carboxy-2-unsubstituted derivatives **8**—**10**. Compound **10** is worthy of note: while lacking 2-carboxylic group of **D** it is able in any case to bind at all three receptor types. This was unexpected because up to now the presence at position-2 of a substituent able to engage either a hydrogen-bond or a lipophylic interaction with the receptor sites was thought to be an important structural requirement for the anchoring of PQZ derivatives at the three receptor types.[15,30] It has to be noted that binding affinities at glycine/NMDA, AMPA and KA receptors of the 2-unsubstituted-1-carboxylic acid **10** are comparable to those of its corresponding 1-unsubstituted-2-carboxylic acid **C**.[21] This finding represents a novelty and indicates that the position of the carboxylate group in the upper region of the PQZ scaffold does not affect anchoring at the receptor sites.

As previously reported,[21,25,28] the presence of a chlorine atom at position-9 of the PQZ framework is an important structural requirement for enhancing glycine/NMDA receptor affinity and selectivity. In fact, the 9-chloro derivatives **6**, **8**, and **10** possess glycine/NMDA receptor affinities and selectivities which are higher than those of their corresponding 9-unsubstituted derivatives **4**, **7**, and **9**.

Finally, affinities of the 1-carboxylic acids **4**—**6, 9**—**10** are generally higher than those of their corresponding esters **1**—**3, 7**—**8**, demonstrating that the presence of a free carboxylate group at position-1 is important for anchoring at all three receptor types.

In conclusion, the synthesis of the herein reported 1-carboxy PQZ derivatives produced new glycine/NMDA receptor antagonists endowed with good affinity and selectivity, and allowed us to further investigate the SAR of tricyclic heteroaromatic systems as iGluR receptor antagonists.

Experimental

Chemistry Silica gel plates (Merk F_{254}) and silica gel 60 (Merck; 70—230 mesh) were used for analytical and column chromatography, respectively. All melting points were determined on a Gallenkamp melting point apparatus. Microanalyses were performed with a Perkin-Elmer 260 elemental analyser for C, H, N. The IR spectra were recorded with a Perkin-Elmer 1420 spectrometer in Nujol mulls and are expressed in cm^{-1}. The ^{1}H-NMR spectra were obtained with a Varian Gemini 200 instrument at 200 MHz. The chemical shifts are reported in δ (ppm) and are relative to the central peak of the solvent, which is always DMSO-d_6. The following abbreviations are used: s=singlet, d=doublet, dd=double doublet, t=triplet, q=quartet, m=multiplet, and br=broad. All the exchangeable protons were confirmed by addition of D_2O. The physical data of new compounds are shown in Table 2.

Table 2. Physical Data of the Newly Synthesised Compounds

Compd.	mp [°C]	Solvent[a)]	Yield [%]
1	283—286	A	85
2	>300	A	90
3	291—294	A	95
4	>300	B	75
5	>300	C	70
6	>300	A	70
9	>300	B	80
10	>300	C	85

a) Recrystallization solvents: A=glacial acetic acid; B=dimethylformamide/water; C=dimethylformamide.

8-Chloro-1-(ethoxycarbonyl)-5-oxo-5,6-dihydro-pyrazolo[1,5-*c*]quinazoline-2-carboxylic Acid (11), and 8,9-Dichloro-1-(ethoxycarbonyl)-5-oxo-5,6-dihydro-pyrazolo[1,5-*c*]quinazoline-2-carboxylic Acid (12) The title compounds were prepared as previously reported.[25]

General Procedure for the Preparation of 2-Carboxyamides 1—3 *N*-3-(Dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride (EDC hydrochloride) (1.64 mmol), 1-hydroxybenzotriazole (HOBt) (1.64 mmol) and the suitable amine (1.64 mmol) were successively added to a solution of **11**, **12** (1.49 mmol) in anhydrous *N*,*N*-dimethylformamide (DMF) (5 ml). The mixture was stirred at room temperature for 12 h and then dilution with 0.1 M HCl (15 ml) afforded a precipitate which was collected by filtration and washed with water. Compounds **1**—**3** displayed the following spectral and analytical data:

Ethyl 8-Chloro-5-oxo-2-(phenylcarbamoyl)-5,6-dihydro-pyrazolo[1,5-*c*]quinazoline-1-carboxylate **1**: ^{1}H-NMR δ: 1.13 (3H, t, J=6.9 Hz), 4.27 (2H, q, J=6.9 Hz), 7.15 (1H, m), 7.40 (4H, m), 7.75 (2H, d, J=7.6 Hz), 8.83 (1H, d, J=8.8 Hz), 10.79 (1H, s), 12.48 (1H, br s) *Anal.* Calcd for $C_{20}H_{15}ClN_4O_4$: C, 58.47; H, 3.69; N, 13.64. Found: C, 58.23; H, 3.50; N, 13.79.

Ethyl 2-(Benzylcarbamoyl)-8-chloro-5-oxo-5,6-dihydropyrazolo[1,5-*c*]quinazoline-1-carboxylate **2**: ^{1}H-NMR δ: 1.18 (3H, t, J=6.9 Hz), 4.26 (2H, q, J=6.9 Hz), 4.45 (2H, d, J=6.2 Hz), 7.33 (7H, m), 8.52 (1H, d, J=9.1 Hz), 9.30 (1H, m), 12.38 (1H, s) *Anal.* Calcd for $C_{21}H_{17}ClN_4O_4$: C, 59.36; H, 4.04; N, 13.19. Found: C, 59.53; H, 4.15; N, 13.23.

Ethyl 8,9-Dichloro-5-oxo-2-(phenylcarbamoyl)-5,6-dihydro-pyrazolo[1,5-*c*]quinazoline-1-carboxylate **3**: ^{1}H-NMR δ: 1.12 (3H, t, J=7.0 Hz), 4.28 (2H, q, J=7.0 Hz), 7.12 (1H, t, J=7.7 Hz), 7.37 (2H, t, J=7.7 Hz), 7.58 (1H, s), 7.74 (2H, d, J=7.7 Hz), 9.21 (1H, s), 10.81 (1H, s), 12.60 (1H, br s) IR cm^{-1}: 3490, 3284, 1755, 1707, 1659. *Anal.* Calcd for $C_{20}H_{14}Cl_2N_4O_4$: C, 53.94; H, 3.18; N, 12.59. Found: C, 53.79; H, 3.19; N, 12.50.

Ethyl 8-Chloro-5-oxo-5,6-dihydro-pyrazolo[1,5-*c*]quinazoline-1-carboxylate (7) and Ethyl 8,9-dichloro-5-oxo-5,6-dihydro-pyrazolo[1,5-*c*]quinazoline-1-carboxylate (8) The title compounds were prepared by heating **11**, **12** in an oil bath over their melting points, according to the procedure reported in ref. 25.

General Procedure for the Preparation of the 1-Carboxylic Acids 4—6, 9, 10 An aqueous solution of KOH (2 M, 3 ml) was added to a suspension of the ethyl 1-carboxylate esters **1**—**3**, **7**, **8** (0.6 mmol) in EtOH (15 ml). The mixture was stirred at room temperature until the disappearance of the starting material (TLC monitoring, eluting system $CHCl_3$/MeOH 9 : 1) and then diluted with water (30 ml). The clear cold (5 °C) solution upon acidification with 6 M HCl afforded a solid which was collected by filtration and washed with water. Compounds **4**—**6**, **9**, **10** displayed the following spectral and analytical data:

8-Chloro-5-oxo-2-(phenylcarbamoyl)-5,6-dihydro-pyrazolo[1,5-*c*]quinazoline-1-carboxylic Acid **4**: ^{1}H-NMR δ: 7.10 (1H, m), 7.37 (4H, m), 7.71 (2H, d, J=8.0 Hz), 8.93 (1H, d, J=9.1 Hz) 10.76 (1H, s), 12.43 (1H, s), 13.52 (1H, br s) *Anal.* Calcd for $C_{18}H_{11}ClN_4O_4$: C, 56.48; H, 2.90; N, 14.64. Found: C, 56.67; H, 2.99; N, 14.70.

2-(Benzylcarbamoyl)-8-chloro-5-oxo-5,6-dihydro-pyrazolo[1,5-*c*]quinazoline-1-carboxylic Acid **5**: ^{1}H-NMR δ: 4.48 (2H, d, J=6.2 Hz), 7.34 (7H, m), 8.82 (1H, d, J=8.4 Hz), 9.42 (1H, t, J=6.2 Hz), 12.37 (1H, s), 13.87 (1H, br s) *Anal.* Calcd for $C_{19}H_{13}ClN_4O_4$: C, 57.51; H, 3.31; N, 14.12. Found: C, 57.44; H, 3.42; N, 14.18.

8,9-Dichloro-5-oxo-2-(phenylcarbamoyl)-5,6-dihydro-pyrazolo[1,5-*c*]quinazoline-1-carboxylic acid **6**: ^{1}H-NMR δ: 7.12 (1H, t, J=7.0 Hz), 7.36 (2H, m), 7.56 (1H, s), 7.73 (2H, d, J=7.2 Hz), 9.32 (1H, s), 10.81 (1H,

s), 12.54 (1H, s) IR cm^{-1}: 3664, 3510, 1767, 1696. *Anal.* Calcd for $C_{18}H_{10}Cl_2N_4O_4$: C, 51.81; H, 2.42; N, 13.43. Found: C, 52.01; H, 2.44; N, 13.36.

8-Chloro-5-oxo-5,6-dihydro-pyrazolo[1,5-*c*]quinazoline-1-carboxylic Acid **9**: ^{1}H-NMR δ: 7.39 (2H, m), 8.40 (1H, s), 9.38 (1H, d, J=8.7 Hz), 12.30 (1H, br s). *Anal.* Calcd for $C_{11}H_6ClN_3O_3$: C, 50.11; H, 2.30; N, 15.94. Found: C, 49.99; H, 2.21; N, 16.01.

8,9-Dichloro-5-oxo-5,6-dihydro-pyrazolo[1,5-*c*]quinazoline-1-carboxylic Acid **10**: ^{1}H-NMR δ: 7.48 (1H, s), 8.39 (1H, s), 9.60 (1H, s), 12.40 (1H, s), 13.35 (1H, br s) IR cm^{-1}: 3170, 1734, 1712. *Anal.* Calcd for $C_{11}H_5Cl_2N_3O_3$: C, 44.32; H, 1.69; N, 14.10. Found: C, 44.21; H, 1.58; N, 14.03.

Pharmacology. Binding Assays Rat cortical synaptic membrane preparation, [^{3}H]glycine and [^{3}H]AMPA binding experiments were performed following the procedure described in refs. 31 and 32, respectively. High-affinity [^{3}H]kainate binding assays were performed on rat cortical membranes according to previously reported methods.[22)]

Sample Preparation and Result Calculation A stock of 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 to four displacement curves based on four to six scalar concentrations of the tested compound in triplicate using the ALLFIT computer program[33)] and, in the case of tritiated glycine and AMPA binding, converted to K_i values by application of the Cheng–Prusoff equation.[34)] In 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.

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