Synthesis and Biological Evaluation of a Series of Quinazoline-2-carboxylic Acids and Quinazoline-2,4-diones as Glycine-NMDA Antagonists: A Pharmacophore Model Based Approach

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Summary

The synthesis and glycine-NMDA binding activity of a series of quinazoline-2-carboxylic acids **1** and quinazoline-2,4-diones **2**, containing all the essential and optional pharmacophoric descriptors required by a putative glycine antagonist model, are reported. The binding results show that only three of the title compounds displayed micromolar receptor affinity, demonstrating how disappointing the synthesis of receptor ligands based only on interaction models can be.

Introduction

Neurodegeneration produced by toxic level of glutamate is now suggested to be a causative factor in the pathology of a number of neurological diseases[1].

The glutamate neurotoxicity is mainly due to the overactivation of ionotropic NMDA and non-NMDA receptors. NMDA stands for *N*-methyl-D-aspartate which is the synthetic agonist of the receptor. The NMDA receptor has multiple ligand recognition sites such as glutamate, polyamines, protons, Zn^{2+}, redox agents and a binding site for glycine, an obligatory coagonist of glutamate. Indeed, glutamate is virtually inactive to promote the opening of the Na^+/Ca^{2+} channel unless the glycine site is also occupied. Blockage of the glycine site by antagonists results in a complete inhibition of NMDA mediate functions. Thus, within recent years, competitive glycine-NMDA antagonists have received considerable attention for their therapeutical potential in neurodegenerative disorders[2–3].

A putative model of the glycine site antagonist pharmacophore, based on structure-activity relationship studies [3] and on superposition of potent and selective antagonists such as 5,7-dichloro kinurenic acid (**A**)[4], 3-(4-hydroxyphenyl)-4-hydroxy-5,7-dichloroquinolin-2(1*H*)-one (**B**)[5], and *trans* 1,2,3,4-tetrahydro-2-carboxy-4-[1-(3-phenyl-2-oxoimidazo lidinyl)]-5,7-dichloroquinoline (**C**)[6], seems to require: a) a flat area binding a nitrogen heteroaromatic moiety, b) a Coulombic interaction with a negatively charged group, and c) a hydrogen donor and acceptor located in the northern and southern regions of the receptor, respectively. Moreover, to enhance the affinity of a ligand two other optional hydrophobic regions must be occupied: a) a large one in the northern or north-eastern area accommodating a lipophilic substituent, and b) two size-limited binding pockets which fit the hydrophobic substituents of the fused benzo ring (see Figure 1).

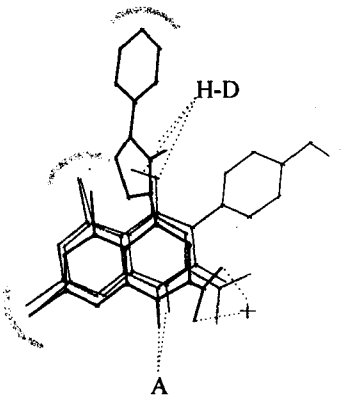
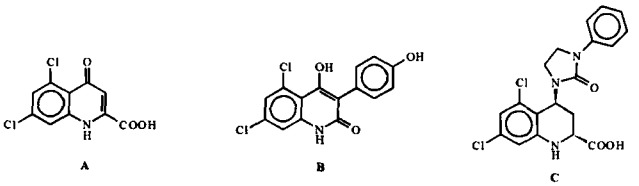


Figure 1. Putative pharmacophore of glycine-NMDA receptor antagonists based on superposition of 5,7-dichlorokinurenic acid[4] (thin line) (**A**), 3-(4-hydroxyphenyl)-4-hydroxy-5,7-dichloroquinolin-2(1*H*)-one[5] (grey line) (**B**), and *trans* 1,2,3,4-tetrahydro-2-carboxy-4-[1-(3-phenyl-2-oxoimidazolidinyl)]-5,7-dichloroquinoline[6] (bold line) (**C**). D-H and A represent a receptor hydrogen bond donor and acceptor, respectively; + represents a positively charged group of the receptor. A bulk tolerance region, accommodating the substituent in the north-eastern or northern side, and two size-limited binding pockets, accommodating the hydrophobic substituents of the fused benzo ring, are also shown.



As a part of a program aimed at finding new antagonists of the glycine-NMDA binding site, we have prepared a series of quinazoline-2-carboxylic acids **1** and quinazoline-2,4-diones **2** which, in our opinion, should contain all the essential and optional pharmacophoric descriptors of a glycine antagonist (see Figure 2).

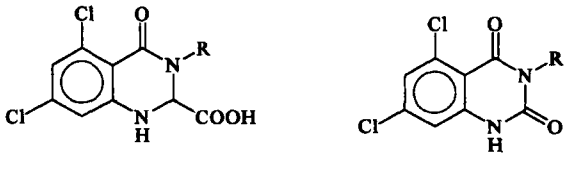


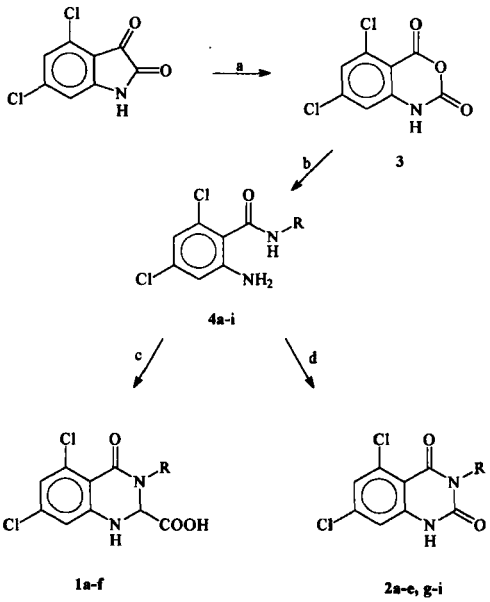
Figure 2

  0365-6233/97/0505-0129 $17.50 +.50/0

Chemistry

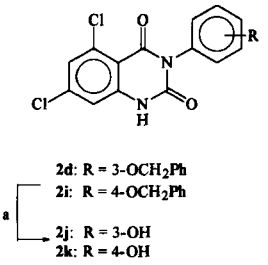
The synthesis of quinazolines of series **1** and **2** is outlined in Schemes 1–3.

Oxidation with chromium trioxide of 4,6-dichloroisatin[7] gave the 1,2-dihydro-5,7-dichloro-3,1-benzoxazine-2,4-dione[8] **3**. The 2-amino-4,6-dichlorobenzamides **4a–i** were prepared by allowing **3** to react with the suitable amine. Compounds **4a–i** were the key intermediates for the synthesis of both series **1** and **2** compounds. In fact, by reacting compounds **4a–f** with glioxylic acid compounds **1a–f** were obtained, while the reaction of **4a–e, g–i** with triphosgene yielded compounds **2a–e, g–i** (see Scheme 1).



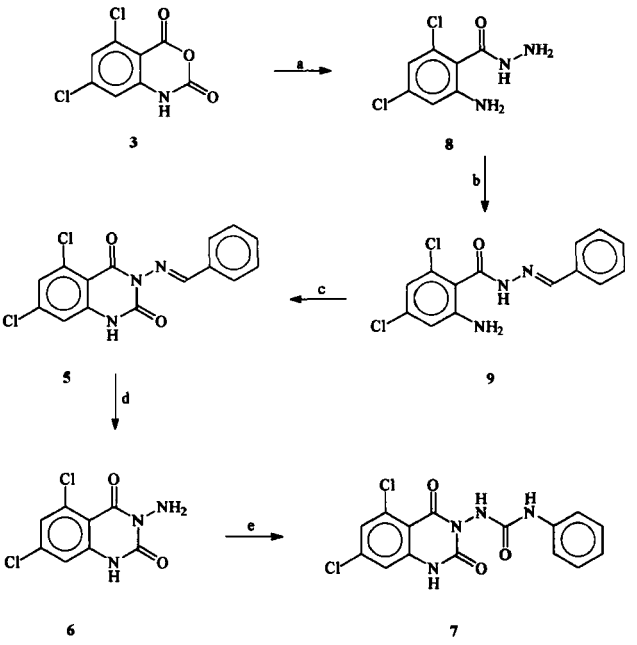
Scheme 1. a: CrO_3. b: RNH_2. c: HCOCOOH. d: $(Cl_3CO)_2CO$.

Catalytic reduction of compounds **2d** and **2i** produced the 3-(*meta*- or *para*-hydroxyphenyl) derivatives **2j** and **2k** (see Scheme 2).



Scheme 2. a: H_2/Pd/C.

In Scheme 3 the synthetic steps which yielded the quinazolin-3-amino derivatives **5–7** are illustrated. 3,1-Benzoxazine **3** was reacted with hydrazine to afford the 2-amino-4,6-dichlorobenzoyl hydrazide **8**, which, by reaction with benzaldehyde, gave the corresponding *N'*-benzylidene hydrazide **9**. Cyclization of **9** with triphosgene produced the 3-(*N*-benzyliden)aminoquinazolinedione **5**. Elimination of the benzylidene residue of **5** afforded the



Scheme 3. a: N_2H_4. b: PhCHO. c: $(Cl_3CO)_2CO$. d: H_2/Pd/C. e: PhNCO.

amine **6**, which, when reacted with phenyl isocyanate, yielded the ureido derivative **7**.

Results and Conclusions

All the reported quinazolines **1a–f**, **2a–e, g–k, 5,** and **7** were tested for their ability to displace [^{3}H]glycine from its specific binding in rat cortical membranes. The binding results show that only three of the tested compounds are able to displace the radioligand binding in the micromolar range (see Table 1), while all the others are inactive up to a concentration of 100 μM.

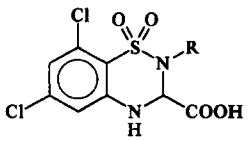
Table 1. Binding activity of quinazoline-2,4-diones **2a, 2j,** and **2k.**[a]

Compd	R	IC_{50} (μM)[b]
2a		23 ± 6
2j		2.5 ± 0.3
2k		3.9 ± 0.4

[a] The tests were carried out using DMSO as solvent. [b] Concentrations necessary for 50% inhibition of [^{3}H]glycine binding are means ±SEM of three separate determinations in triplicate.

These very disappointing results cannot be explained, especially for the quinazoline-2-carboxylic acids **1a–f**, which contain the negatively charged 2-carboxylic group necessary for the Coulombic interaction and thought to be the most

important feature for glycine antagonists. In fact, a number of bicyclic heteroaromatic 2-carboxylic acid derivatives[4, 9–10] are reported to be selective antagonists at the glycine site. Among these bicyclic 2-carboxylic acid derivatives there are the benzothiadiazine 1,1-dioxides reported by the Rhone-Poulenc-Rorer researchers (RPR ligands)[10].



RPR ligands

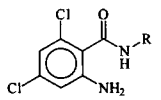
R = aryl, aralkyl

Figure 3

RPR ligands, which are bioisosters of compounds **1a–f** and display high glycine-NMDA affinity, demonstrate that there is no hindrance between the carboxylic group and the adjacent aryl substituent which can both be accommodated on the receptor recognition site[2].

Successively, we went on with our search for glycine-NMDA receptor antagonists taking as lead one of the templates shown in Figure 1, namely the 3-(4-hydroxyphenyl)-4-hydroxy-5,7-dichloroquinolin-2(1*H*)-one[5], which displayed nanomolar binding activity. We therefore synthesised the quinazoline-2,4-diones **2a–e, g–k** in which the ion-ion interaction between the 2-carboxylic acid and the positively charged group of the glycine receptor site was replaced by a dipole-ion interaction. In fact, in the 2-carbonyl group the electrons are delocalized on the more negative oxygen atom,

Table 2. Physical data for the intermediates **4a–i**.

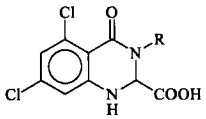


4a–i

Comp.	R	Mp (°C)	Solv[a]	Yield (%)
4a		143–144	A	78
4b		218–219	B	90
4c		204–205	C	74
4d		95–98	A	80
4e		109–110	A	55
4f		132–134	D	80
4g		146–147	A	62
4h		100–102	A	58
4i		172–174	D	68

[a] Purification solvents: A = carbon tetrachloride. B = ethyl acetate. C = ethanol. D = cyclohexane/ethyl acetate.

Table 3. Physical data for quinazoline-2-carboxylic acids **1a–f** [a].

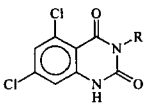


1a-f

Comp	R	Mp (°C)	Yield (%)
1a		227 dec	60
1b		165 dec	80
1c		125 dec	55
1d		227 dec	50
1e		200 dec	60
1f		200 dec	75

[a] All the compounds were purified by acid/base exchange as described in the Experimental Section.

Table 4. Physical data for quinazoline-2,4-diones **2a–e, g–k**.



2a-e, g-k

Comp	R	Mp (°C)	Solv[a]	Yield (%)
2a		316-318	A	77
2b		305-306	A	80
2c		>320	B	78
2d		264-265	B	85
2e		280-281	B	87
2g		309-310	B	89
2h		275-276	A/B	93
2i		283-284	B	95
2j		>310	B	60
2k		>310	B	72

[a] Purification solvents: A = ethanol. B = glacial acetic acid.

Table 5. Spectroscopic data of compounds **4a–i**, **1a–f**, and **2a–e, g–k**.

Compd	IR	^{1}H NMR[a]
4a	3500, 3400, 3280, 1650	A: 5.75 (br s, 2H, NH_2), 6.73–6.76 (m, 2H, ar), 7.14 (t, 1H, ar, J = 7.2 Hz), 7.35 (t, 2H, ar, J = 8.0 Hz), 7.72 (d, 2H, ar, J = 7.4 Hz), 10.49 (s, 1H, NH)
4b	3300, 3260, 1680	A: 2.28 (s, 3H, CH_3), 5.73 (br s, 2H, NH_2), 6.73–6.76 (m, 2H, ar), 7.15 (d, 2H, ar, J = 8.6 Hz), 7.61 (d, 2H, ar, J = 8.6 Hz), 10.39 (s, 1H, NH)
4c	3500, 3380, 3280, 1630	A: 3.74 (s, 3H, OCH_3), 5.72 (br s, 2H, NH_2), 6.73–6.75 (m, 2H, ar), 6.91 (d, 2H, ar, J = 9.0 Hz), 7.62 (d, 2H, ar, J = 9.0 Hz), 10.33 (s, 1H, NH)
4d	3480, 3360, 3300, 1650	B: 4.71 (br s, 2H, NH_2), 5.04 (s, 2H, CH_2), 6.52 (d, 1H, ar, J = 1.7 Hz), 6.71–6.81 (m, 2H, ar), 7.08–7.42 (m, 8H, ar), 8.08 (s, 1H, NH)
4e	3460, 3300, 1650	A: 4.44 (d, 2H, CH_2, J = 5.9 Hz), 5.56 (br s, 2H, NH_2), 6.67–6.71 (m, 2H, ar), 7.24–7.36 (m, 5H, ar), 8.97 (t, 1H, NH, J = 5.9 Hz)
4f	3460, 3360, 3320, 1630	A: 4.45 (d, 2H, CH_2, J = 4.4 Hz), 5.58 (br s, 2H, NH_2), 6.69–6.72 (m, 2H, ar), 7.30–7.60 (m, 4H, ar), 9.03 (t, 1H, NH, J = 5.8 Hz).
4g	3500, 3400, 3300, 1650	B: 2.36 (s, 3H, CH_3), 4.83 (br s, 2H, NH_2), 6.64 (d, 1H, ar, J = 1.6 Hz), 6.78 (d, 1H, ar, J = 1.6 Hz), 6.99–7.47 (m, 4H, ar), 7.64 (br s, 1H, NH)
4h	3500, 3400, 3300, 1650	B: 3.83 (s, 3H, OCH_3), 6.71–6.77 (m, 3H, ar), 7.06–7.40 (m, 3H, ar), 7.72 (br s, 1H, NH)
4i	3500, 3400, 3280, 1640	A: 5.12 (s, 2H, CH_2), 5.71 (br s, 2H, NH_2), 6.75 (d, 2H, ar, J = 8.8 Hz), 7.01 (d, 2H, ar, J = 8.8 Hz), 7.32–7.47 (m, 5H, ar), 7.63 (d, 2H, ar, J = 9.1 Hz), 10.36 (s, 1H, NH)
1a	3400–2500, 1730, 1660	A: 5.64 (d, 1H, H-2, J = 4.0 Hz), 6.92 (d, 1H, ar, J = 2.1 Hz), 7.01 (d, 1H, ar, J = 2.1 Hz), 7.31–7.49 (m, 5H, ar), 8.22 (d, 1H, NH, J = 2.1 Hz), 13.50 (br s, 1H, COOH)
1b	3400–2500, 1740, 1660	A: 5.58 (d, 1H, H-2, J = 3.9 Hz), 6.91 (d, 1H, ar, J = 2.0 Hz), 7.01 (d, 1H, ar, J = 2.0 Hz), 7.20–7.35 (m, 5H, ar), 8.19 (d, 1H, NH, J = 3.9 Hz), 13.52 (br s, 1H, COOH)
1c	3660, 3540, 3460, 3290, 1740, 1650	A: 3.78 (s, 3H, OCH_3), 5.53 (d, 1H, H-2, J = 3.1 Hz), 6.89–7.00 (m, 4H, ar), 7.30 (d, 2H, ar, J = 9.1 Hz), 8.16 (d, 1H, NH, J = 3.1 Hz), 13.61 (br s, 1H, COOH)
1d	3300–2600, 1730, 1660	A: 5.12 (s, 2H, CH_2), 5.64 (d, 1H, H-2, J = 3.9 Hz), 6.91–7.06 (m, 5H, ar), 7.31–7.52 (m, 6H, ar), 8.22 (d, 1H, NH, J = 3.9 Hz), 13.41 (br s, 1H, COOH)
1e	3380, 3300–2600, 1760, 1630	A: 4.17 (d, 1H, benzyl proton, J = 15.2 Hz), 5.15 (d, 1H, H-2, J = 3.1 Hz), 5.23 (d, 1H, benzyl proton, J = 15.2 Hz), 6.85–6.87 (m, 2H, ar), 7.25–7.38 (m, 5H, ar), 7.95 (d, 1H, NH, J = 3.1 Hz), 13.40 (br s, 1H, COOH)
1f	3250, 3200–2500, 1720, 1650	A: 4.26 (d, 1H, benzyl proton, J = 15.7 Hz), 5.13 (d, 1H, benzyl proton, J = 15.7 Hz), 5.33 (d, 1H, H-2, J = 3.7 Hz), 6.88–6.91 (m, 2H, ar), 7.27–7.54 (m, 4H, ar), 8.05 (d, 1H, NH, J = 3.7 Hz), 13.41 (br s, 1H, COOH)
2a	3200, 1740, 1680	A: 7.22–7.51 (m, 7H, ar), 11.75 (s, 1H, NH)
2b	3200, 1740, 1690	A: 2.38 (s, 3H, CH_3), 7.21–7.42 (m, 6H, ar), 11.76 (s, 1H, NH)
2c	3200, 1740, 1690	A: 3.81 (s, 3H, OCH_3), 7.04 (d, 2H, ar, J = 8.9 Hz), 7.20–7.24 (m, 3H, ar), 7.41 (d, 1H, ar, J = 1.8 Hz), 11.75 (s, 1H, NH)
2d	3200, 1740, 1680	A: 5.10 (s, 2H, CH_2), 6.88–7.50 (m, 11H, ar), 11.80 (s, 1H, NH)
2e	3200, 1730, 1680	A: 5.02 (s, 2H, CH_2), 7.19–7.41 (m, 7H, ar), 11.79 (s, 1H, NH)
2g	3200, 1740, 1680	A: 2.36 (s, 3H, CH_3), 7.08–7.43 (m, 6H, ar), 11.79 (s, 1H, NH)
2h	3210, 1790, 1670	A: 3.77 (s, 3H, OCH_3), 6.86–7.04 (m, 3H, ar), 7.22 (d, 1H, ar, J = 1.9 Hz), 7.35–7.42 (m, 2H, ar), 11.75 (s, 1H, NH)
2i	3200, 1740, 1670	A: 5.17 (s, 2H, CH_2), 7.06–7.51 (m, 11H, ar), 11.61 (s, 1H, NH)
2j	3520, 3420, 3200, 1740, 1670	A: 6.69–6.84 (m, 3H, ar), 7.20–7.29 (m, 2H, ar), 7.39 (d, 1H, ar, J = 1.9 Hz), 9.62 (s, 1H, OH), 11.75 (s, 1H, NH)
2k	3540, 3440, 3200, 1760, 1660	A: 6.82 (d, 2H, ar, J = 8.7 Hz), 7.06 (d, 2H, ar, J = 8.7 Hz), 7.21 (d, 1H, ar, J = 2.1 Hz), 7.40 (d, 1H, ar, J = 2.1 Hz), 9.64 (s, 1H, OH), 11.65 (s, 1H, NH) .

[a]Solvents: A = DMSO-d_6. B = $CDCl_3$.

which in this way, functioning as a dipole, may interact with the positively charged group of the receptor as shown in Figure 1.

This working hypothesis was confirmed by the binding activity of the first compound of the series, i.e. the 3-phenyl derivative **2a**, which displayed an $IC_{50} = 23$ μM.

In the hope of improving the glycine-NMDA receptor affinity of **2a**, a range of substituents were introduced at position-3. However, *meta* or *para*-phenyl substituents (compounds **2b–d, g–i**), whatever their nature or steric hindrance, produced a drop in receptor affinity. The replacement of the phenyl of **2a** with a benzyl group (**2e**) also gave a decrement of affinity.

On the contrary, the presence on the 3-appended phenyl of a hydroxy group either at the *meta* or *para* position (**2j** and **2k**, respectively) strongly improved the receptor affinity. This may be attributed either to the acidic nature of the phenol substituent or to the presence, in the large hydrophobic pocket which accommodates the 3-hydrophobic group, of a hydrogen bonding site. In effect, the improved binding activity of *meta* or *para*-hydroxyphenyl derivatives has already been reported[5, 11–12].

Other attempts to enhance the affinity of **2a**, by spacing the quinazoline moiety from the phenyl ring with a benzylidenamino (compound **5**) or an ureido group (**7**), resulted in a loss of binding activity.

In conclusion, although the compounds studied contain all the features required by the antagonist pharmacophore model, they show a very poor affinity for glycine-NMDA receptors. These results underline the limits of the interaction model and seem to make its critical revision necessary.

Acknowledgements

Valuable assistance in animal handling was provided by M.G. Giovannini.

Experimental Section

A) Chemistry

Silica gel plates (Merck F_{254}) and silica gel 60 (Merck; 70–230 mesh) were used for analytical and column chromatography, respectively. All melting points were determined on a Gallenkamp melting point apparatus. Microanalyses were performed with a Perkin-Elmer 260 elemental analyser for C, H, N, and the results were within ±0.4% of the theoretical values. The IR spectra were recorded with a Perkin-Elmer 1420 spectrometer in Nujol mulls and are expressed in cm^{-1}. The ^1H 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. The following abbreviations are used: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad, and ar = aromatic protons. Physical data for the newly synthesised compounds are listed in Tables 2–4 while spectroscopic data are shown in Table 5.

The molecular geometry of the template antagonists[4–6] shown in Figure 1 were minimised by the MOPAC 6.0 semiempirical MO package[13] using AM1 parametrization running on an IBM RISC 6000 3CT workstation. Conformational analysis of *trans* 1,2,3,4-tetrahydro-2-carboxy-4-[1-(3-phenyl-2-oxoimidazolidinyl)]-5,7-dichloroquinoline[6] was performed using a systematic search on the "search compare" module of the Insight II 95.0 program.

1,2-Dihydro-5,7-dichloro-3,1-benzoxazine-2,4-dione **3**[8]

Chromium trioxide (13.8 mmol) was added portionwise to a hot (90 °C) suspension of 4,6-dichloroisatin[7] (13.8 mmol) in glacial acetic acid (15 mL) and acetic anhydride (15 mL). The mixture was heated (90 °C) for 30 min. Addition of water (60–80 mL) yielded a solid which was collected, washed with water and recrystallized. Mp 266–268 °C (dec.) (methanol), 75% yield. ^1H NMR (DMSO-d_6): 7.12 (d, 1H, ar, $J = 1.9$ Hz), 7.51 (d, 1H, ar, $J = 1.9$ Hz), 11.91 (s, 1H, NH).

General Procedure for the Preparation of 2-Amino-4,6-dichlorobenzamides **4a–i**

A solution of the suitable amine (3 mmol) in glacial acetic acid (5 mL) was slowly added (30 min) to a hot (100 °C) solution of **3** (3 mmol) in glacial acetic acid (10 mL). The mixture was heated (100 °C) for 5–10 min. Evaporation of the solvent at reduced pressure afforded a residue which, in all cases but two (**4d** and **4h**), was treated with carbon tetrachloride (10–15 mL), filtered and recrystallized. Purification of the residue of **4d** and **4h** required column chromatography, using the cyclohexane/ethyl acetate (6:4) system as eluent. Evaporation at reduced pressure of the solvent of the first eluates yielded a residue which was recrystallized.

General Procedure for the Preparation of (±)1,2,3,4-Tetrahydro-5,7-dichloro-4-oxoquinazoline-2-carboxylic Acids **1a–f**

An excess of glyoxylic acid monohydrate (usually 4.5 mmol, 9 mmol in the case of **4a**) was added to a solution of **4a–f** (3 mmol) in anhydrous benzene (30 mL). The solution was heated (50 °C) under nitrogen atmosphere. The reaction was monitored by TLC and the heating was continued until the starting material disappeared. Upon cooling, the mixture yielded a solid which was collected, washed with water and purified by acid/base exchange as follows: the crude product was treated with sodium carbonate (10%, 10 mL), the insoluble was filtered off and the clear solution was acidified with hydrochloric acid (6 N) to yield a solid which was collected, washed with water and dried.

General Procedure for the Preparation of 1,2,3,4-Tetrahydro-5,7-dichloroquinazoline-2,4-diones **2a–e, g–i**

Triphosgene (0.6 mmol) and triethylamine (3.6 mmol) were added to a solution of **4a–e, g–i** (1.5 mmol) in anhydrous tetrahydrofuran (10 mL). The mixture was stirred at room temperature for 30–45 min and then diluted with water (30–50 mL) to yield a solid which was collected and recrystallized.

General Procedure for the Preparation of 1,2,3,4-Tetrahydro-5,7-dichloro-3-(meta- or para-hydroxyphenyl)quinazoline-2,4-diones **2j** *and* **2k**

Pd/C (10%, 0.1 g) was added to a warm solution of **2d** or **2i** (1.2 mmol) in glacial acetic acid (150 mL). The mixture was hydrogenated in a Parr apparatus at 30 psi for 4 h. Elimination of the catalyst and evaporation at reduced pressure of the solvent afforded a residue which was recrystallized.

2-Amino-4,6-dichlorobenzoyl Hydrazide **8**

Hydrazine hydrate (55%, 4 mL) was poured into the stirred solid **3** (13 mmol). The mixture was heated (50 °C) for 15 min and then diluted with water (50 mL). The solid was collected and used without further purification. Mp 160–162 °C, 45% yield. ^1H NMR (DMSO-d_6): 4. 40 (br s, 2H, NH_2), 5.60 (br s, 2H, NH2), 6.61–6.70 (m, 2H, ar), 9.51 (br s, 1H, NH). IR: 3380, 3300, 3220, 1610, 1580.

N'-Benzyliden-N-(2-amino-4,6-dichlorobenzoyl) Hydrazide **9**

Benzaldehyde (3.2 mmol) was added to a suspension of **8** (3.2 mmol) in absolute ethanol (10 mL). The mixture was refluxed for 1 h. Evaporation at reduced pressure of the solvent yielded a residue which was treated with diethyl ether, filtered and recrystallized. Mp 185–188 °C (cyclohexane/ethyl acetate), 85% yield. ^1H NMR (CD_3OD): 6.73–6.77 (m, 2H, ar), 7.40–7.46 (m, 3H, ar), 7.79–7.83 (m, 2H, ar), 8.18 (s, 1H, CH). IR: 3480, 3360, 3200, 1640.

1,2,3,4-Tetrahydro-3-(N-benzyliden)amino-5,7,-dichloroquinazoline-2,4-dione **5**

The title compound was obtained from **9** (1.5 mmol), triphosgene (0.6 mmol) and triethylamine (3.6 mmol) following the procedure described to prepare **2a–e, g–i**. Mp 278–280 °C (acetone), 60% yield. ^{1}H NMR (DMSO-d_6): 7.25 (d, 1H, ar, J = 1.3 Hz), 7.47–7.68 (m, 4H, ar), 7.90–7.98 (m, 2H, ar), 8.73 (s, 1H, CH), 11.98 (s, 1H, NH). IR: 3220, 3200, 1740, 1720, 1650.

1,2,3,4-Tetrahydro-3-amino-5,7-dichloroquinazoline-2,4-dione **6**

Pd/C (10%, 0.1 g) was added to a solution of **5** (1.5 mmol) in glacial acetic acid (100 mL). The mixture was hydrogenated in a Parr apparatus at 20 psi for 2 h. Elimination of the catalyst and evaporation at reduced pressure of the solvent yielded a residue which was treated with ethanol, filtered and recrystallized. Mp 278–280 °C (methanol), 50% yield. ^{1}H NMR (DMSO-d_6): 5.48 (s, 2H, NH_2), 7.19 (d, 1H, ar, J = 1.9 Hz), 7.41 (d, 1H, ar, J = 1.9 Hz), 12.01 (br s, 1H, NH). IR: 3400, 3340, 3250, 1730, 1680.

1,2,3,4-Tetrahydro-3-(N'-phenylureido)-5,7-dichloroquinazoline-2,4-dione **7**

A solution of phenyl isocyanate (0.7 mmol) in anhydrous benzene (3 mL) was added dropwise to a suspension of **6** (0.7 mmol) in anhydrous benzene (20 mL). The mixture was refluxed under nitrogen atmosphere for 2 h. The solid was collected, washed with water and recrystallized. Mp >310 °C (methanol), 70% yield. ^{1}H NMR (DMSO-d_6): 6.96 (t, 1H, ar, J = 7.1 Hz), 7.21–7.33 (m, 3H, ar), 7.40–7.48 (m, 3H, ar), 8.63 (s, 1H, NH), 9.18 (s, 1H, NH), 11.54 (br s, 1H, NH). IR: 3400, 3100, 1740, 1700, 1670.

B) Biochemistry

Crude synaptic membranes were prepared from cerebral cortices of male Sprague-Dawley rats (170–250 g). The tissue was homogenised in 20 vol of ice-cold 0.32 M sucrose, containing 20 µg/ml phenylmethanesulfonyl fluoride, using a glass-Teflon homogenizer (clearance = 0.15–0.23 mm). The homogenate was centrifuged at 1000 × g for 10 min and the resulting supernatant further centrifuged at 20000 × g for 20 min. The final pellet was resuspended in 20 vol of ice-cold distilled water, dispersed with an Ultra-Turrax sonicator (30% of maximum speed) for 30 s and centrifuged at 8000 × g for 20 min. The supernatant and the soft upper layer of the pellet were collected together and centrifuged at 48000 × g for 20 min. The membranes were resuspended once more in distilled water, centrifuged and frozen at –80 °C.

On the day of the experiment, appropriate amounts of membranes were thawed at room temperature, resuspended (0.5 mg protein/ml) in cold 0.05 M Tris acetate buffer (pH 7.0) containing 0.08 % *v/v* Triton X-100, and stirred for 10 min at 0–2 °C. The membranes were then collected by centrifugation at 20000 × g for 10 min, submitted to four additional resuspension and centrifugation cycles with fresh buffer and finally resuspended in cold Tris acetate buffer to yield 0.2–0.3 mg protein/assay tube. [^{3}H]Glycine (51.3 Ci/mmol, New England Nuclear) binding assays were carried out in ice for 30 min at 10 nM ligand concentration in a total 1.0 ml vol. Bound radioactivity was separated by rapid filtration through Whatman GF/B discs using a Millipore filtration apparatus. Non-specific binding was determined in the presence of 1 mM D-serine. The IC_{50} values were calculated from displacement curves based on 4–6 scalar concentrations of the test compounds in triplicate, using the ALLFIT computer program[14]. A stock 1 mM solution of the test compounds was prepared in 50% dimethyl sulfoxide (DMSO). Subsequent dilutions were accomplished in buffer. DMSO up to a final 2% concentration was seen to affect [^{3}H]glycine binding negligibly.

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