Eur. J. Med. Chem. 36 (2001) 203–209

PII: S0223-5234(00)01203-4/FLA

Short communication

Synthesis of a set of ethyl 1-carbamoyl-3-oxoquinoxaline-2-carboxylates and of their constrained analogue imidazo[1,5-a]quinoxaline-1,3,4-triones as glycine/NMDA receptor antagonists

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Received 19 July 2000; revised 7 November 2000; accepted 23 November 2000

Abstract – The synthesis and glycine/NMDA and AMPA receptor affinities of a set of ethyl (±) 1-*N*-carbamoyl-1,2,3,4-tetrahydro-3-oxoquinoxaline-2-carboxylates **1**–**11** and those of their constrained analogue (±) 1,2,3,3a,4,5-hexahydroimidazo[1,5-a]quinoxaline-1,3,4-triones **12**–**24** are reported. Compounds **1**–**11** bear a side-chain at position 1 which has been spatially constrained in compounds **12**–**24**. Most of the reported tricyclic derivatives **12**–**24** showed glycine/NMDA binding activity comparable to that of their corresponding bicyclic analogues **1**–**11** providing further evidence that the spatial orientation of the side-chain is an important structural requirement for glycine/NMDA receptor antagonists. © 2001 Éditions scientifiques et médicales Elsevier SAS

quinoxaline derivatives / glycine/NMDA receptor / AMPA receptor / antagonists

1. Introduction

Ionotropic glutamate receptors (iGluRs), namely *N* - methyl - D - aspartate (NMDA), (RS) - 2 - amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)propionic acid (AMPA) and kainic acid (KA), play key roles in neuronal transmission in the mammalian central nervous system, but are also likely to be involved in numerous pathological and excitotoxic processes [1, 2]. Selective iGluR agonists and antagonists have provided the tools for understanding the physiological and pathological roles of each receptor type [2].

Like other ligand-gated ion channels, the NMDA receptor is modulated by a number of different molecules and among them the amino acid glycine, binding at the strychnine-insensitive binding site (glycine/NMDA), is particularly important as a co-agonist of glutamate for NMDA receptor activation [3].

Structure–activity relationship studies on quinoxaline-2,3-diones, which have been reported to have glycine/NMDA and AMPA receptor antagonist activities [4–7], have revealed that there are remarkable structural similarities in the binding sites of both receptor types [8, 9]. As shown in *figure 1*, both glycine/NMDA and AMPA antagonists possess a NH proton donor which binds to a proton acceptor of the receptors, as well as negatively-charged heteroatoms able to form a Coulombic interaction with a positive site of the receptors. Both glycine/NMDA and AMPA receptors can tolerate a polar side-chain called X (*figure 1*), and both prefer to accommodate electron-withdrawing group(s) at R_1 and/or R_2, although some quinoxaline-2,3-diones with $R_1 = R_2 =$ Me have been reported to bind at both receptors with low micromolar affinity [10]. However, glycine/NMDA or AMPA selectivity can be modulated by

Abbreviations: AMPA, (RS)-2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl) propionic acid; DMSO, dimethylsulfoxide; glycine/NMDA, strychnine-insensitive binding site; iGluRs, ionotropic glutamate receptors; NMDA, *N*-methyl-D-aspartate.

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varying the nature of R_1, R_2 and the spatial orientation of the X polar side-chain. Generally, bulky electron-withdrawing NO_2, CF_3 or Br substituents and a nitrogen-containing heterocycle are preferred by the AMPA receptor in R_1 and R_2, respectively, while the glycine/NMDA site preferably accommodates chlorine atoms in R_1 and R_2. Moreover, the AMPA receptor prefers polar side-chains oriented towards the benzo-fused moiety (i.e. north-western orientation), while the glycine/NMDA one accommodates polar side-chains on the opposite side (i.e. north-eastern orientation) [6].

On this basis, the present paper reports the synthesis and glycine/NMDA and AMPA receptor-binding activity of a set of ethyl (±) 1-*N*-carbamoyl-1,2,3,4-tetrahydro-3-oxoquinoxaline-2-carboxylates **1**–**11** and of their constrained analogue (±) 1,2,3,3a,4,5-hexahydroimidazo[1,5-a]quinoxaline-1,3,4-triones **12**–**24** (*figure 2*).

The tetrahydro-3-oxoquinoxaline-2-carboxylates **1**–**11** can be regarded as analogues of the 3-nitro-3,4-dihydro-2(1*H*)-quinolones [11] which are selective glycine/NMDA receptor antagonists. In fact, the electron-withdrawing 2-carboxylate group of compounds **1**–**11**, like the 3-nitro one of the quinolone analogues, confers acidity to the 2-hydrogen atom, increasing the charge density on the lactam oxygen at position-3. Moreover, compounds **1**–**11** can be easily transformed by cyclization into compounds **12**–**24**, where the possibility of delocalization of the negative charge increases the charge density, not only on the 4-lactam oxygen, but also on the 3-oxo function. Finally, the tricyclic derivatives **12**–**24** offer a further advantage over the corresponding bicyclic analogues **1**–**11** by bearing a side-chain constrained in the north-eastern region which could improve the glycine/NMDA receptor affinity.

2. Chemistry

The target compounds **1**–**24** were obtained starting from the 3-oxoquinoxaline-2-carboxylates **25**–**27**. The unsubstituted-3-oxoquinoxaline-2-carboxylate **25** and its 6,7-dimethyl-substituted analogue **27** were prepared following a reported method [12]. The synthesis of the unknown ethyl (±) 6-chloro-1,2,3,4-tetrahydro-3-oxoquinoxaline-2-carboxylate **26** is illustrated in *figure 3*. Briefly, catalytic reduction of the diethyl *N*-[(2,4-dinitrophenyl)amino]malonate **28** [13] yielded the ethyl 6-amino-3,4-dihydro-3-oxoquinoxaline-2-

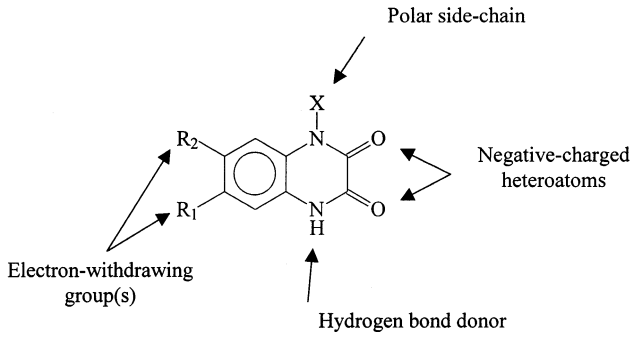


Figure 1. Common structural requirements for glycine/NMDA and AMPA receptor antagonists.

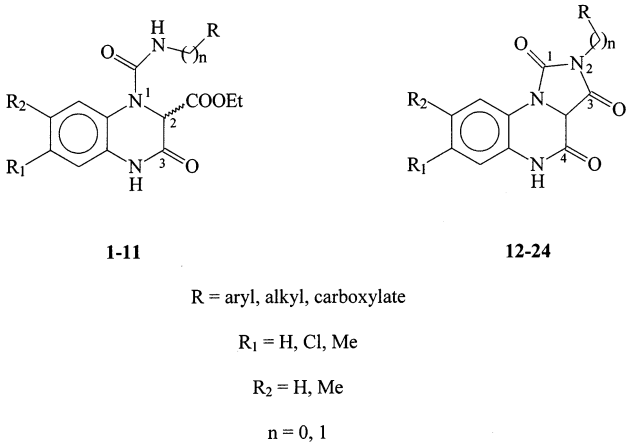


Figure 2. The newly synthesized compounds.

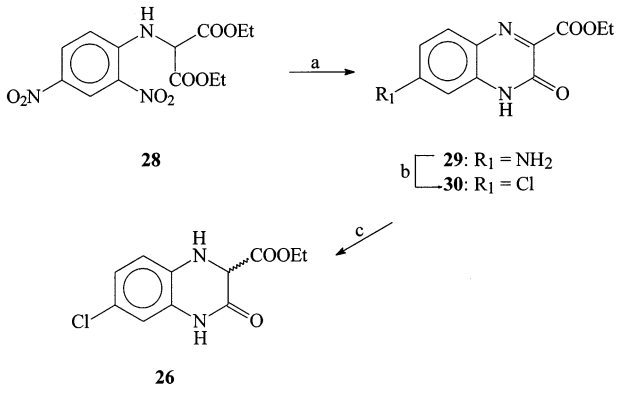


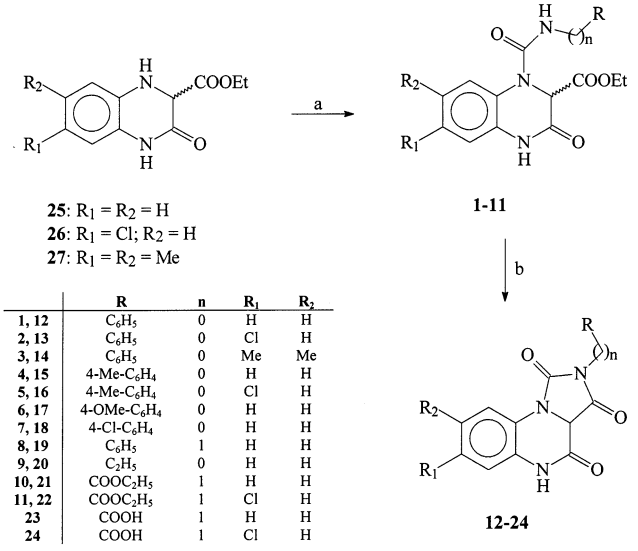
Figure 3. Synthesis of ethyl (±) 6-chloro-1,2,3,4-tetrahydro-3-oxoquinoxaline-2-carboxylate **26**. (a) H_2/Pd/C; (b) $CuCl_2$, *t*-BuONO; (c) $Na_2S_2O_4$.

carboxylate **29** which was transformed into its corresponding 6-chloro-analogue **30** with $CuCl_2$ and *t*-butyl nitrite. Reduction of **30** with sodium hydrosulfite yielded the tetrahydro-derivative **26**.

Allowing **25**–**27** to react with isocyanates, the ethyl (±) 1-*N*-carbamoyl-3-oxoquinoxaline-2-carboxylates **1**–**11** were prepared (*figure 4*). By heating **1**–**11** with diluted HCl the tricyclic 2-aryl- **12**–**18**, 2-benzyl- **19**, 2-ethyl- **20** and 2-acetic acid derivatives **23**–**24** were obtained. The 2-acetic acid derivatives **23**–**24** resulted from cyclization and hydrolysis of the corresponding bicyclic esters **10**–**11**. The acetic esters **21** and **22** were prepared by heating their corresponding bicyclic analogues **10** and **11** over their melting points.

3. Results and discussion

Compounds **1**–**24** were tested for their ability to displace both tritiated glycine and AMPA from their specific binding in rat cortical membranes.The binding data, shown in *table I*, indicated that compounds **1**–**24** bind to the glycine/NMDA receptor with low micromolar affinity while only three compounds, namely **2**, **11** and **24**, displayed micromolar AMPA affinity.



25: $R_1 = R_2 = H$
26: $R_1 = Cl$; $R_2 = H$
27: $R_1 = R_2 = Me$

	R	n	R_1	R_2
1, 12	C_6H_5	0	H	H
2, 13	C_6H_5	0	Cl	H
3, 14	C_6H_5	0	Me	Me
4, 15	4-Me-C_6H_4	0	H	H
5, 16	4-Me-C_6H_4	0	Cl	H
6, 17	4-OMe-C_6H_4	0	H	H
7, 18	4-Cl-C_6H_4	0	H	H
8, 19	C_6H_5	1	H	H
9, 20	C_2H_5	0	H	H
10, 21	$COOC_2H_5$	1	H	H
11, 22	$COOC_2H_5$	1	Cl	H
23	COOH	1	H	H
24	COOH	1	Cl	H

Figure 4. Synthesis of ethyl (±) 1-*N*-carbamoyl-1,2,3,4-tetrahydro-3-oxoquinoxaline-2-carboxylates **1**–**11** and (±) 1,2,3,3a,4,5-hexahydroimidazo[1,5-a]quinoxaline-1,3,4-triones **12**–**24**. (a) $R(CH_2)_nNCO$; (b) 2 N HCl or heating above melting point.

The 1-*N*-phenylcarbamoyl derivative **1** and its 2-phenyl constrained analogue **12** displayed comparable glycine/NMDA receptor affinity ($K_i = 57.3$ and 48.5 μM, respectively), indicating that a polar side-chain bearing a lipophilic phenyl moiety in the north-eastern region of the molecules is recognized by the glycine/NMDA receptor. Introduction of an electron-withdrawing chlorine atom at position 6 of **1** and at position 7 of **12** yielded compounds **2** and **13**, respectively, which showed a 17-fold enhanced glycine/NMDA affinity with respect to their corresponding unsubstituted derivatives (**1** and **12**). In agreement with the literature data [11], the presence of two electron-donating methyl groups in R_1 and R_2 are tolerated by the glycine/NMDA receptor (see **3** and **14** *vs.* **1** and **12**, respectively). However, compounds **3** and **14** were 26- and 10-fold less active than their corresponding chloro-substituted derivatives **2** and **13**, respectively.

The introduction of a para-substituent on the phenyl ring of the parent compounds **1**–**2** yielded the bicyclic derivatives **4**–**7** which were all, except for the 6-chloro-1-*N*-(4-methylphenyl)- derivative **5** ($K_i = 5.2$ μM), inactive at the glycine/NMDA receptor. On the contrary, all the para-substituted tricyclic derivatives **15**–**18** bind to the glycine/NMDA receptor with comparable or higher affinities than their 2-phenyl-unsubstituted derivatives **12**–**13**.

Introduction of a methylene spacer between the carbamoyl or the tricyclic core and the phenyl moiety (as in the benzyl derivatives **8** and **19)** into compounds **1** and **12**, and the replacement of their lipophilic phenyl ring with the less lipophilic ethyl group (as in the ethyl derivatives **9** and **20**) dramatically affected glycine/NMDA receptor affinity.

Finally, the lipophilic phenyl moiety of **1**–**2** and **12**–**13** was replaced with an acetate or an acetic acid group yielding compounds **10**–**11** and **21**–**24,** respectively. It was, in fact, demonstrated that in bicyclic and tricyclic derivatives of similar size and shape a carbethoxy or a free carboxylic group yielded potent glycine/NMDA receptor antagonists [14–17]. In our case, compounds bearing an acetate or an acetic acid group showed an affinity comparable to that of the corresponding phenyl-substituted derivatives only when a chloro substituent on the

Table I. Glycine/NMDA and AMPA binding activity.

Compd.	R	n	R_1	R_2	K_i (μM) or % inhibition[a]	
					[^{3}H]Glycine	[^{3}H]AMPA
1	C_6H_5	0	H	H	57.3 ± 9.0	21%
2	C_6H_5	0	Cl	H	3.4 ± 0.8	61.3 ± 18.1
3	C_6H_5	0	Me	Me	88.3 ± 15.0	20%
4	4-Me-C_6H_4	0	H	H	34%	30%
5	4-Me-C_6H_4	0	Cl	H	5.2 ± 0.8	30%
6	4-OMe-C_6H_4	0	H	H	26%	15%
7	4-Cl-C_6H_4	0	H	H	35%	5%
8	C_6H_5	1	H	H	0%	16%
9	C_2H_5	0	H	H	23%	25%
10	$COOC_2H_5$	1	H	H	97.0 ± 8.1	17%
11	$COOC_2H_5$	1	Cl	H	3.1 ± 0.6	72.1 ± 12
12	C_6H_5	0	H	H	48.5 ± 7.9	28%
13	C_6H_5	0	Cl	H	2.8 ± 0.5	42%
14	C_6H_5	0	Me	Me	20.2 ± 1.9	45%
15	4-Me-C_6H_4	0	H	H	10.9 ± 2.5	21%
16	4-Me-C_6H_4	0	Cl	H	5.0 ± 1.1	24%
17	4-OMe-C_6H_4	0	H	H	45.5 ± 7.5	0%
18	4-Cl-C_6H_4	0	H	H	15.0 ± 1.8	15%
19	C_6H_5	1	H	H	0%	24%
20	C_2H_5	0	H	H	10%	17%
21	$COOC_2H_5$	1	H	H	30%	0%
22	$COOC_2H_5$	1	Cl	H	4.1 ± 0.7	40%
23	COOH	1	H	H	71.2 ± 12.1	26%
24	COOH	1	Cl	H	2.2 ± 0.3	51.1 ± 4

[a] K_i values are means ± S.E.M. of 3–4 separate determinations in triplicate or percentage of inhibition (I%) of specific binding at 100 μM concentration.

benzo-fused moiety was present (compare compounds **11** and **22**, **24** with **2** and **13**, respectively).

Some selected compounds of our two series were shown to inhibit the binding of the NMDA receptor channel-blocking agent [^{3}H]-(+)-MK-801 to rat cortical membranes incubated with 10 μM glutamate and 0.1 μM glycine (*table II*). The IC_{50} values of these compounds for glutamate-stimulated [^{3}H]-(+)-MK-801 binding showed a trend similar to that of their K_i values for [^{3}H]-glycine binding, thus indicating that these new ligands behave like weak glycine/NMDA receptor antagonists [18–20].

In conclusion, the synthesis and glycine/NMDA receptor affinity of the ethyl (±) 1-*N*-carbamoyl-1,2,3,4-tetrahydro-3-oxoquinoxaline-2-carboxylates **1**–**11** and of their constrained tricyclic analogues **12**–**24** provide further evidence that the spatial orientation of the polar side-chain in the north-eastern region of the molecule combined with the presence of a chlorine atom in R_1 are two important requirements to obtain glycine/NMDA receptor antagonists.

Table II. Inhibition of stimulated [H^3]-(+)-MK-801 binding.

Compd.	[^{3}H]-(+)-MK-801 IC_{50} (μM)[a]
2	14.6 ± 2.1
5	23.9 ± 1.2
11	32.0 ± 5.0
12	45.0 ± 3.0
13	18.0 ± 1.1
15	21.0 ± 3.0
16	36.8 ± 4.2
17	61.0 ± 7.0
22	19.3 ± 1.5
24	20.5 ± 1.6

[a] Concentrations giving 50% inhibition of stimulated [^{3}H]-(+)-MK-801 binding. All assays were carried out in the presence of 10 μM glutamate and 0.1 μM glycine. The results were calculated from 3–4 separate determinations in triplicate.

4. Experimental protocols

4.1. Chemistry

Silica gel plates (Merck F_{254}) were used for analytical chromatography. All melting points were determined on a Gallenkamp melting point apparatus. Microanalyses were performed with a Perkin-Elmer 260 elemental analyzer for C, H, N, and the results were within ±0.4% of the theoretical values. 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. The following abbreviations are used: s, singlet; d, doublet; dd, double doublet; t, triplet; q, quartet; m, multiplet; ar, aromatic protons and br, broad. Physical and analytical data of the newly synthesized compounds are listed in *table III*; the legend also reports the spectral data of some significant compounds.

4.1.1. Ethyl 6-amino-3,4-dihydro-3-oxoquinoxaline-2-carboxylate **29**

A mixture of diethyl *N*-[(2,4-dinitrophenyl)-amino]malonate **28** [13] (1.47 mmol) and Pd/C (10%, 0.06 g) in absolute ethanol (100 mL) was hydrogenated in a Parr apparatus at 30 psi for 12 h. Elimination of the catalyst yielded a solution which was further stirred at room temperature for another 12 h yielding a green suspension. Evaporation of the solvent at reduced pressure yielded a green solid which was washed with diethyl ether and recrystallized.

4.1.2. Ethyl 6-chloro-3,4-dihydro-3-oxoquinoxaline-2-carboxylate **30**

Compound **29** (1.07 mmol) was slowly (1 h) added to a cooled (0–5 °C) mixture of $CuCl_2$ (1.34 mmol) and *tert*-butyl nitrite (1.60 mmol) in acetonitrile (20 mL). The mixture was allowed to stand for 90 min. Then HCl (6 N, 20 mL) was added to the mixture which was extracted with ethyl acetate (20 mL×3). The combined organic extracts were washed with water (20 mL×3) and dried (Na_2SO_4). Evaporation at reduced pressure of the solvent yielded a solid which was treated with diethyl ether, collected and recrystallized.

4.1.3. Ethyl (±) 6-chloro-1,2,3,4-tetrahydro-3-oxoquinoxaline-2-carboxylate **26**

An excess of $Na_2S_2O_4$ (9.3 mmol) was added to a suspension of **30** (3.72 mmol) in ethanol/water (1:1, 20 mL). The mixture was heated at 80 °C for 90 min. The mixture was cooled and the resulting solid was collected and washed with water. The title compound could not be recrystallized because it easily dehydrogenated to lead compound **30**. Nevertheless, compound **26** was pure enough to be characterized and used in the next preparation without further purification.

4.1.4. General procedure for the preparation of ethyl (±) 1-N-carbamoyl-1,2,3,4-tetrahydro-3-oxoquinoxaline-2-carboxylates **1**–**9** and (±) N-(2-carboxyethyl-1,2,3,4-tetrahydro-3-oxoquinoxalinyl-1-N-carbonyl)glycine ethyl esters **10**–**11**

A solution of **25**–**27** [12] (3.63 mmol) and the suitable isocyanate (18 mmol) in anhydrous benzene (40 mL) was heated at reflux under nitrogen atmosphere until the disappearance of the starting material (TLC monitoring). The cooled mixture yielded a solid which was collected, thoroughly washed with diethyl ether and recrystallized.

4.1.5. General procedure for the preparation of (±) 1,2,3,3a,4,5-hexahydroimidazo[1,5-a]quinoxaline-1,3,4-triones **12**–**20** and (±) 1,2,3,3a,4,5-hexahydro-1,3,4-trioxoimidazo[1,5-a]quinoxalin-2-acetic acids **23**–**24**

A mixture of **1**–**11** (1.47 mmol) in HCl (2 N, 10 mL) was heated at 110 °C for 2 h. The cooled mixture yielded a solid which was collected, washed with water and then recrystallized.

4.1.6. General procedure for the preparation of ethyl (±) 1,2,3,3a,4,5-hexahydro-1,3,4-trioxoimidazo[1,5-a]quinoxaline-2-acetates **21**–**22**

Compound **10** or **11** (0.7 mmol) was heated over its melting point for 30 min. The cooled mass was treated with diethyl ether, collected and recrystallized.

4.2. Biochemistry

Rat cortical synaptic membrane preparation, [^{3}H]glycine and [^{3}H]AMPA binding experiments were performed according to previously described procedures [21, 22].

4.2.1. [^{3}H]-(+)-MK-801 binding

[^{3}H]-(+)-MK-801 ((+)-5-methyl-10,11-dihydro-5*H*-dibenzo-[a,d]cyclohepten-5,10-imine maleate) binding assays were carried out according to Yoneda and Ogita [23] with slight modifications. Preparations of frozen rat cortical membranes were resuspended (0.5 mg protein

Table III. Physical and analytical data of newly synthesized compounds.

Compd.	R	n	R_1	R_2	m.p., °C	Cryst. solv.[a]	% Yield	Analysis (C, H, N)
1	C_6H_5	0	H	H	196–198	A	80	$C_{18}H_{17}N_3O_4$
2[b]	C_6H_5	0	Cl	H	210–212	A	60	$C_{18}H_{16}ClN_3O_4$
3	C_6H_5	0	Me	Me	200–204	A	70	$C_{20}H_{21}N_3O_4$
4	4-Me-C_6H_4	0	H	H	198–199	A	85	$C_{19}H_{19}N_3O_4$
5	4-Me-C_6H_4	0	Cl	H	206–208	B	35	$C_{19}H_{18}ClN_3O_4$
6	4-OMe-C_6H_4	0	H	H	204–207	A	90	$C_{19}H_{19}N_3O_5$
7	4-Cl-C_6H_4	0	H	H	206–208	A	90	$C_{18}H_{16}ClN_3O_4$
8	C_6H_5	1	H	H	186–188	A	85	$C_{19}H_{19}N_3O_4$
9[c]	C_2H_5	0	H	H	169–171	C	60	$C_{14}H_{17}N_3O_4$
10	$COOC_2H_5$	1	H	H	169–172	A	80	$C_{16}H_{19}N_3O_6$
11[d]	$COOC_2H_5$	1	Cl	H	172–174	A	60	$C_{16}H_{18}ClN_3O_6$
12	C_6H_5	0	H	H	256–259	A	85	$C_{16}H_{11}N_3O_3$
13[e]	C_6H_5	0	Cl	H	269–272	A	70	$C_{16}H_{10}ClN_3O_3$
14	C_6H_5	0	Me	Me	>300	D	80	$C_{18}H_{15}N_3O_3$
15	4-Me-C_6H_4	0	H	H	278–279	E	80	$C_{17}H_{13}N_3O_3$
16	4-Me-C_6H_4	0	Cl	H	>300	D	80	$C_{17}H_{12}ClN_3O_3$
17	4-OMe-C_6H_4	0	H	H	263–265	A	80	$C_{17}H_{13}N_3O_4$
18	4-Cl-C_6H_4	0	H	H	294–297	A	90	$C_{16}H_{10}ClN_3O_3$
19	C_6H_5	1	H	H	277–280	D	90	$C_{17}H_{13}N_3O_3$
20[f]	C_2H_5	0	H	H	272–274	A	50	$C_{12}H_{11}N_3O_3$
21	$COOC_2H_5$	1	H	H	220–222	A	50	$C_{14}H_{13}N_3O_5$
22[g]	$COOC_2H_5$	1	Cl	H	236–238	B	40	$C_{14}H_{12}ClN_3O_5$
23	COOH	1	H	H	>300	F	40	$C_{12}H_9N_3O_5$
24[h]	COOH	1	Cl	H	263–266 dec	A	60	$C_{12}H_8ClN_3O_5$
26[i]			Cl	H	196–201		50	
29[j]			NH_2		260–264 dec	A	80	$C_{11}H_{11}N_3O_3$
30[k]			Cl		235–237	B	50	$C_{11}H_9ClN_2O_3$

[a] Recrystallization solvents: A, ethanol; B, ethyl acetate; C, cyclohexane/ethyl acetate; D, nitromethane; E, glacial acetic acid; F, dimethylformamide/water.

[b] ^{1}H-NMR (DMSO-d_6): 1.10 (t; 3H, CH_3, $J = 7.0$ Hz); 4.06–4.15 (m; 2H, CH_2); 5.49 (s; 1H, H-2); 7.03–7.14 (m; 3H, ar); 7.27–7.50 (m; 5H, ar); 9.37 (s; 1H, carbamoyl NH); 11.11 (s; 1H, NH at position-4).

[c] ^{1}H-NMR (DMSO-d_6): 1.02–1.12 (m; 6H, $2CH_3$); 3.08–3.17 (m; 2H, CH_2); 4.04–4.08 (m; 2H, CH_2); 5.52 (s; 1H, H-2); 6.97–7.11 (m; 4H, 3 ar + carbamoyl NH); 7.45 (d; 1H, ar, $J = 6.0$ Hz); 10.90 (s; 1H, NH at position-4).

[d] ^{1}H-NMR (DMSO-d_6): 1.05 (t; 3H, CH_3, $J = 6.2$ Hz); 1.19 (t; 3H, CH_3, $J = 7.0$ Hz); 3.78–4.11 (m; 6H, $3CH_2$); 5.46 (s; 1H, H-2); 6.98 (s; 1H, ar); 7.11 (d; 1H, ar, $J = 8.7$ Hz); 7.15–7.61 (m; 2H, 1 ar + carbamoyl NH); 11.05 (s; 1H, NH at position-4).

[e] ^{1}H-NMR (DMSO-d_6): 5.39 (s; 1H, H-3a, it exchanges with D_2O); 7.02 (d; 1H, ar, $J = 2.2$ Hz); 7.15 (dd; 1H, ar, $J = 8.7$, 2.2 Hz); 7.39–7.52 (m; 5H, ar); 7.65 (d; 1H, ar, $J = 8.7$ Hz); 10.98 (s; 1H, NH).

[f] ^{1}H-NMR (DMSO-d_6): 1.13 (t; 3H, CH_3, $J = 7.1$ Hz); 3.45 (q; 2H, CH_2, $J = 7.1$ Hz); 5.23 (s; 1H, H-3a, it exchanges with D_2O); 6.95–7.15 (m; 3H, ar); 7.61 (d; 1H, ar, $J = 7.3$ Hz); 10.83 (s; 1H, NH).

[g] ^{1}H-NMR (DMSO-d_6): 1.20 (t; 3H, CH_3, $J = 7.0$ Hz); 4.10–4.27 (m; 4H, $2CH_2$); 5.49 (s; 1H, H-3a, it exchanges with D_2O); 7.01 (d; 1H, ar, $J = 2.2$ Hz); 7.15 (dd; 1H, ar, $J = 8.7$, 2.2 Hz); 7.59 (d; 1H, ar, $J = 8.7$ Hz); 11.05 (s; 1H, NH).

[h] ^{1}H-NMR (DMSO-d_6): 4.15 (s; 2H, CH_2); 5.47 (s; 1H, H-3a, it exchanges with D_2O); 7.01 (d; 1H, ar, $J = 2.2$ Hz); 7.15 (dd; 1H, ar, $J = 8.5$, 2.2 Hz); 7.60 (d; 1H, ar, $J = 8.5$ Hz); 11.05 (s; 1H, NH).

[i] This compound easily dehydrogenated, thus it could not be recrystallized. Nevertheless, it was pure enough to be characterized. ^{1}H-NMR (DMSO-d_6): 1.16 (t; 3H, CH_3, $J = 7.1$ Hz); 4.11 (q; 2H, CH_2, $J = 7.1$ Hz); 4.60 (d; 1H, H-2, $J = 2.1$ Hz); 6.74–6.87 (m; 4H, 3 ar + NH at position-1); 10.68 (s; 1H, NH at position-4).

[j] ^{1}H-NMR (DMSO-d_6): 1.29 (t; 3H, CH_3, $J = 7.1$ Hz); 4.28 (q; 2H, CH_2, $J = 7.1$ Hz); 6.32 (d; 1H, ar, $J = 2.2$ Hz); 6.47 (s; 2H, NH_2); 6.63 (dd; 1H, ar, $J = 8.8$, 2.2 Hz); 7.44 (d; 1H, ar, $J = 8.8$ Hz); 12.28 (s; 1H, NH).

[k] ^{1}H-NMR (DMSO-d_6): 1.34 (t; 3H, CH_3, $J = 7.1$ Hz); 4.39 (q; 2H, CH_2, $J = 7.1$ Hz); 7.35–7.45 (m; 2H, ar); 7.87 (d; 1H, ar, $J = 8.7$ Hz); 12.93 (s; 1H, NH).

mL^{-1}) in ice-cold 5 mM Tris-HCl buffer, pH 7.4, containing 0.08% v/v Triton X-100 and stirred for 10 min at 0–2 °C. They were then collected by centrifugation (48 000 × g for 10 min) and submitted to four additional resuspension and centrifugation cycles before finally being resuspended in the appropriate volume of buffer

(0.2–0.3 mg protein/tube) for the binding assay. The assay incubations were carried out at room temperature for 120 min with 2.5 nM [^{3}H]-(+)-MK-801 (22.5 Ci mmol^{-1}), 10 μM glutamic acid and 0.1 μM glycine in the presence and absence of the test compound in a total volume of 0.5 mL. Bound radioactivity was separated by filtration through GF/C filters presoaked in 0.05% polyethylenimmine and washed with ice-cold buffer (3× 5 mL). Non-specific binding was determined in the presence of 100 μM phencyclidine hydrochloride.

4.2.2. Sample preparation and result calculation

A 1 mM stock solution of the test compound was prepared in 50% dimethylsulfoxide (DMSO). Subsequent dilutions were accomplished in buffer. The IC_{50} values were calculated from 3–4 displacement curves based on 4–6 scalar concentrations of the test compound in triplicate using the ALLFIT computer program [24] and, in the case of tritiated glycine and AMPA binding, converted to K_i values by application of the Cheng-Prusoff equation [25]. In our experimental conditions the dissociation constants (K_D) for [^{3}H]glycine (10 nM) and [^{3}H]AMPA (8 nM) were 75±6 and 28±3 nM, respectively.

Acknowledgements

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

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