Synthesis, Glycine/NMDA and AMPA Binding Activity of Some New 2,5,6-Trioxopyrazino[1,2,3-*de*]quinoxalines and of Their Restricted Analogs 2,5-Dioxo- and 4,5-Dioxoimidazo[1,5,4-*de*]quinoxalines

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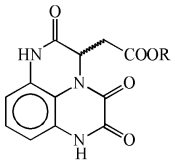
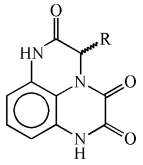
***Key Words**: 2,5,6-Trioxopyrazino[1,2,3-de]quinoxalines; 2,5-dioxoimidazo[1,5,4-de]quinoxalines; 4,5-dioxoimidazo[1,5,4-de]quinoxalines; glycine/NMDA and AMPA binding activity*

Summary

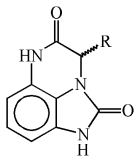
The synthesis of some new 1,2,3,5,6,7-hexahydro-2,5,6-trioxopyrazino[1,2,3-*de*]quinoxalines **1c–g** and of their restricted analogs 2,4,5,6-tetrahydro-2,5-dioxo-1*H*- **2a–g** and 5,6-dihydro-4,5-dioxo-4*H*-imidazo[1,5,4-*de*]quinoxalines **3a–d** is reported. Compounds **1c–g, 2a–g**, and **3a–d** were tested for their binding activity at the glycine/NMDA and AMPA receptors. The results show that only the 6,6,6-tricyclic derivatives **1c–g** are able to bind to the glycine/NMDA and AMPA receptors, although with lower affinity than the previously reported lead compounds **1a–b**. In contrast, the 5,6,6-tricyclic derivatives **2a–g** are inactive at both receptors and only one 4,5-dioxoimidazoquinoxaline (**3b**) displays a weak glycine/NMDA receptor affinity.



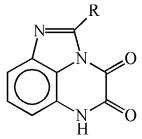
1a: R = Et
1b: R = H



1c–g



2a–g



3a–d

Chart 1

Introduction

NMDA and AMPA receptor subtypes are the best studied among the ionotropic glutamate receptors. AMPA receptors are responsible for most of the fast excitatory synaptic responses involved in neuronal transmission, while NMDA receptors are associated with slow, long-lasting currents which mediate neuronal plasticity and activity [1–2]. Overstimulation of both these receptors by excessive endogenous glutamate occurs in several pathological conditions [3].

The NMDA receptor, like other ligand-gated ion channels, is modulated by a number of molecules acting at different allosteric sites and the amino acid glycine (glycine/NMDA) is particularly important among them for its unique glutamate co-agonist action in channel gating [4]. Antagonists at both glycine/NMDA and AMPA receptors are of great interest for their potential therapeutic use [5–6]. Compounds with combined glycine/NMDA and AMPA receptor antagonist activities have been reported [7–8], leading to the identification of the common structural requirements for receptor-ligand interaction [3, 6, 9].

As a part of a program aimed at finding new antagonists of the glycine/NMDA binding site, we recently reported the synthesis and glycine/NMDA binding activity of ethyl (±) 1,2,3,5,6,7-hexahydro-2,5,6-trioxopyrazino[1,2,3-*de*]quinoxalin-3-acetate **1a** and its corresponding acid **1b** [10] (Chart 1). In the present paper we report the synthesis of other pyrazinoquinoxaline derivatives **1c–g** and of their restricted analogs imidazo[1,5,4-*de*]quinoxaline-2,5-diones **2a–g** and imidazo[1,5,4-*de*]quinoxaline-4,5-diones **3a–d**. Compounds **1c–g**, **2a–g** and **3a–d**, together with the previously reported **1a–b** were tested for their ability to displace radiolabeled glycine and AMPA from their specific binding in rat cerebral membranes. The synthesis of the 5,6,6-tricyclic 2,5-diones **2a–g** and 4,5-diones **3a–d** was performed to assess the importance of the quinoxalinedione and pyrazinone moieties, respectively, in the anchoring of the parent 2,5,6-trioxopyrazinoquinoxalines **1a–g** to the glycine/NMDA and/or AMPA recognition sites.

Chemistry

The synthesis of compounds **1c–g**, **2a–g**, and **3a–d** is shown in Schemes 1–3. Briefly, by reacting the 2,6-dinitrochlorobenzene [11] with (±) phenylglycine or (±) glutamic acid following the method described to prepare **4** [10], the racemic *N*-(2,6-dinitrophenyl)phenylglycine **5** and *N*-(2,6-dinitrophenyl)glutamic acid **6** were obtained, respectively (Scheme 1). Compounds **4–6** were transformed into the corresponding esters **7a** [10], **c–d** which by double catalytic reduction (Pd/C and then Ni/Raney) yielded the 3-substituted-quinoxalines **8a** [10], **c–d**. The latter, by reaction with oxalyl chloride, as described for the preparation of **1a** [10], yielded the pyrazinoquinoxaline-2,5,6-triones **1c–d**. Moreover, the reaction of

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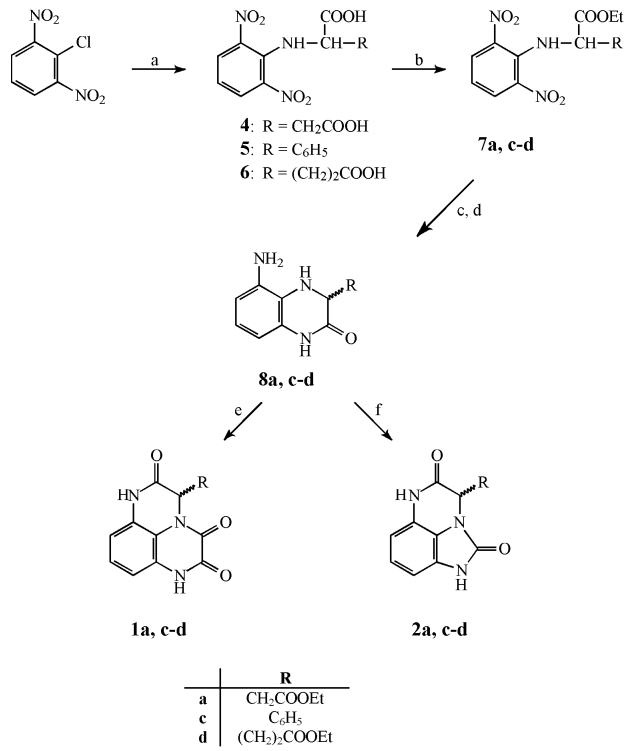
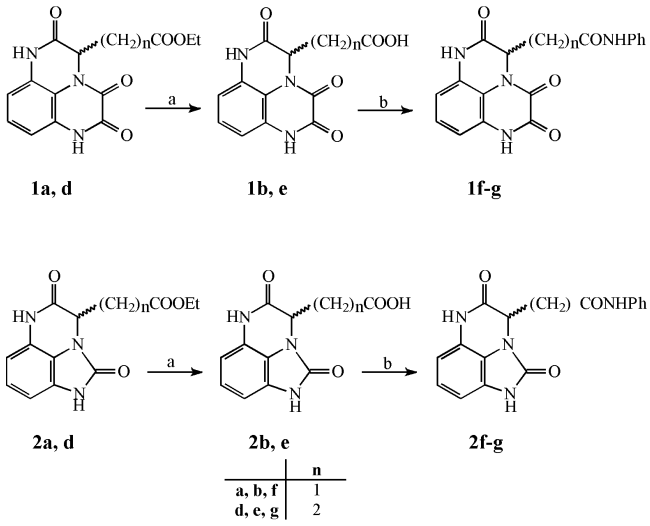


Scheme 1. Reagents: a) $RCH(NH_2)COOH$, $NaHCO_3$. b) conc H_2SO_4, EtOH. c) H_2/Pd/C, conc HCl. d) H_2/Ni-Raney. e) $(COCl)_2$, Et_3N. f) $(Cl_3CO)_2CO$, Et_3N.

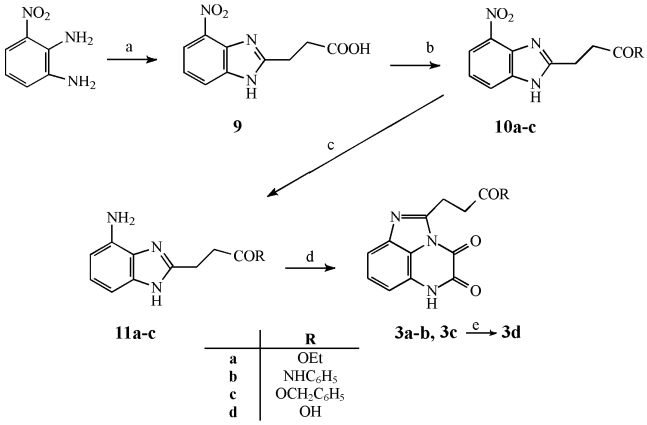


Scheme 2. Reagents: a) 1M NaOH. b) $PhNH_2$, 1-hydroxy-1*H*-benzotriazole, 1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide hydrochloride.

8a, c–d with triphosgene to prepare the imidazoquinoxaline-2,5-diones **2a, c–d** is also illustrated in Scheme 1.

The pyrazinoquinoxaline esters **1a, d** and imidazoquinoxaline esters **2a, d** were hydrolyzed to the corresponding acids **1b, e** and **2b, e** which were transformed into the carboxyamides **1f–g** and **2f–g** (Scheme 2).

Scheme 3 describes the synthetic pathway which yielded the imidazoquinoxaline-4,5-diones **3a–d**. Allowing the 3-nitrophenylene-1,2-diamine to react with succinic anhydride,



Scheme 3. Reagents: a) succinic anhydride, xylene. b) to prepare **10a**: conc H_2SO_4, EtOH; to prepare **10b**: $PhNH_2$, 1-hydroxy-1H-benzotriazole, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride; to prepare **10c**: K_2CO_3, C_6H_5-CH_2Cl. c) to prepare **11a–b**: H_2/Pd/C; to prepare **11c**: $TiCl_3$. d) $(COCl)_2$, Et_3N. e) H_2/Pd/C.

the 4-nitrobenzimidazole-2-propionic acid **9** was prepared. The acid **9** was transformed into the ester **10a** which was catalytically reduced to the 4-amino ester **11a**. The latter was cyclized with oxalyl chloride to the ethyl 4,5-dioxoimidazo[1,5,4-*de*]quinoxaline-2-propionate **3a**. The instability in acidic medium of the ester **3a** prevented its use to obtain other derivatives. Thus, the key intermediate **9** was reacted with aniline by using a combination of 1-(dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride and 1-hydroxy-1*H*-benzotriazole to obtain the 4-nitrocarboxyamide **10b**. Catalytic reduction of **10b** yielded the 4-amino **11b** which was cyclized with oxalyl chloride to **3b**. Finally, the 4-nitro acid **9** was reacted with benzyl chloride to give **10c** which was reduced with $TiCl_3$ to **11c**. Cyclization of **11c** yielded **3c**. The latter was catalytically reduced to afford the 4,5-dioxoimidazo[1,5,4-*de*]quinoxaline-2-propionic acid **3d**.

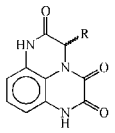
Results and Conclusions

The ability of compounds **1c–g**, **2a–g** and **3a–d** to displace radiolabeled glycine and AMPA from their specific binding in rat brain synaptic membrane preparation, expressed as K_i values, is listed in Table 1. The K_i values at the glycine/NMDA and AMPA receptors of the previously reported **1a–b** [10] have also been calculated and included in Table 1 together with the IC_{50} values at these same receptors of the well known antagonist **PNQX** (1,4,7,8,9,10-hexahydro-9-methyl-6-nitropyrido[3,4-*f*]quinoxalin-2,3-dione [6]).

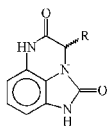
The results show that only the newly reported 2,5,6-trioxopyrazino-quinoxalines **1c–g** display some glycine/NMDA and AMPA receptor affinities. In fact, the 2,5-dioxoimidazo-quinoxalines **2a–g** are completely inactive at both receptors, while only one 4,5-dioxoimidazoquinoxaline, i.e. **3b**, displays a weak affinity for the glycine/NMDA receptor.

However, the newly prepared 6,6,6-tricyclic derivatives **1c–g** show a significant loss of affinity at the glycine/NMDA receptor with respect to that of the previously reported **1a**. In fact, the acetate side chain at position-3 of **1a** seems to be the best one and all changes such as its homologation (**1d**) or

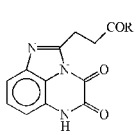
Table 1. Binding activity of tricyclic compounds.



1a–g



2a–g



3a–d

Comp	R	[^{3}H]Gly K_i (μM)[a]	[^{3}H]AMPA K_i(μM)[a]
1a	CH_2COOEt	2.3 ± 0.7	> 100
1b	CH_2COOH	25.6 ± 1.8	49.7 ± 9.3
1c	C_6H_5	> 100	> 100
1d	$(CH_2)_2COOEt$	> 100	> 100
1e	$(CH_2)_2COOH$	37.1 ± 11.5	19.6 ± 2.4
1f	$CH_2CONH\ C_6H_5$	58.2 ± 15	65 ± 7.6
1g	$(CH_2)_2CONH\ C_6H_5$	88 ± 7.1	> 100
2a	CH_2COOEt	> 100	> 100
2b	CH_2COOH	> 100	> 100
2c	C_6H_5	> 100	> 100
2d	$(CH_2)_2COOEt$	> 100	> 100
2e	$(CH_2)_2COOH$	> 100	> 100
2f	$CH_2CONH\ C_6H_5$	> 100	> 100
2g	$(CH_2)_2CONH\ C_6H_5$	> 100	> 100
3a	OEt	> 100	> 100
3b	$NH\ C_6H_5$	61.8 ± 13	> 100
3c	$OCH_2\ C_6H_5$	> 100	> 100
3d	OH	> 100	> 100
PNQX[b]		0.37	0.063 ± 0.012

[a]The values are ± SEM of 3–4 separate determinations in triplicate.
The tested compounds were dissolved in DMSO.
[b]IC_{50} values from Ref. 6.

replacement with a phenyl ring (**1c**) resulted in a significant loss of glycine/NMDA receptor affinity. Moreover, in the 3-acetate series **1a–b, f**, the ester **1a** is active only at the glycine/NMDA receptor, while the acid **1b** and the anilide **1f** show a weak affinity at both receptors. It is however intriguing to note that in the 3-propionate series **1d–e, g**, the ester **1d** and the anilide **1g** are devoid of binding activity at both receptors while the acid **1e** displays some glycine/NMDA and AMPA receptor affinity.

The inactivity at both receptor sites of the five-membered fused-ring 2,5-dioxoimidazo-quinoxalines **2a–g** may be attributed to the lack of the crucial quinoxalinedione moiety[6,8]. The binding results of 5,6,6-tricyclic-4,5-dioxo-imidazoquinoxalines **3a–d** do not allow any conclusion about the importance of the pyrazinone moiety. In fact, by comparing the glycine/NMDA and AMPA binding activities of compounds **3a–b, d** with those of their enlarged analogs **1d**, **1g**, and **1e**, the similar affinity trend of the esters **3a** and **1d** and that of the anilides **3b** and **1g** contrasts with the inactivity of the acid **3d** with respect to the activity of its corresponding propionic acid **1e**.

In conclusion, the synthesis of the 6,6,6-tricyclic derivatives **1c–g** and of their restricted analogs **2a–g** and **3a–d** have led to some weak glycine/NMDA and AMPA receptor ligands, although the binding results do not encourage further investigations of these tricyclic systems.

Acknowledgement

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

Experimental Section

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 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, br = broad, and ar = aromatic protons. Physical and ^{1}H NMR data for the newly synthesized compounds are listed in Tables 2–3.

(±) N-(2,6-Dinitrophenyl)phenylglycine **5** *and (±) N-(2,6-Dinitrophenyl)glutamic Acid* **6**

The title compounds were obtained from the corresponding amino acid (13.2 mmol) and 2,6-dinitrochlorobenzene (12 mmol) as described for the synthesis of **4** [10].

Ethyl (±) N-(2,6-Dinitrophenyl)phenylglycinate **7c** *and Diethyl (±) N-(2,6-Dinitrophenyl)glutamate* **7d**

The title compounds were obtained from **5–6** (7.4 mmol) as described for the synthesis of **7a**[10].

(±) 1,2,3,4-Tetrahydro-5-amino-3-phenylquinoxalin-2-one **8c** *and Ethyl (±) 1,2,3,4-Tetrahydro-5-amino-2-oxoquinoxaline-3-propionate* **8d**

Conc HCl (1.2 mL) and Pd/C (10%, 0.3 g) were added to a solution of **7c–d** (7.55 mmol) in absolute EtOH (200 mL). The mixture was hydrogenated in a Parr apparatus at 30 psi for 12 h. Elimination of the catalyst and evaporation at room temperature and reduced pressure of the solvent yielded a residue which was dissolved in H_2O (70 mL). The solution was neutralized with a saturated solution of $NaHCO_3$ and then extracted with ethyl acetate (3 × 40 mL).The organic layers were dried (Na_2SO_4) and evaporated at room temperature and reduced pressure to yield an oil which was dissolved in EtOH (350 mL). Ni/Raney (50% slurry in H_2O, 1 g) was added to the ethanolic solution which was then hydrogenated in a Parr apparatus at 35 psi for 24 h. Elimination of the catalyst and evaporation at room temperature and reduced pressure of the solvent yielded a residue which became solid with Et_2O.

(±) 1,2,3,5,6,7-Hexahydro-3-phenylpyrazino[1,2,3-de]quinoxaline-2,5,6-trione **1c** *and Ethyl (±) 1,2,3,5,6,7-Hexahydro-2,5,6-trioxopyrazino[1,2,3-de]quinoxaline-3-propionate* **1d**

The title compounds were obtained from **8c–d** (2 mmol) as described for the synthesis of **1a** [10].

(±) Ethyl 2,4,5,6-Tetrahydro-2,5-dioxo-1H-imidazo[1,5,4-de]quinoxalin-4-acetate **2a,** *(±) 2,4,5,6-Tetrahydro-4-phenyl-1H-imidazo[1,5,4-de]quinoxaline-2,5-dione* **2c** *and (±) Ethyl 2,4,5,6-Tetrahydro-2,5-dioxo-1H-imidazo[1,5,4-de]quinoxaline-4-propionate* **2d**

Triphosgene (1.8 mmol) and triethylamine (9 mmol) were added to a solution of **8a, c–d** (4.5 mmol) in anhydrous tetrahydrofuran (THF)

(40 mL).The mixture was stirred at room temperature and under nitrogen atmosphere until disappearance of the starting material (TLC monitoring). Dilution with H_2O (30 mL) afforded a solid which was collected and washed with Et_2O.

(±) 1,2,3,5,6,7-Hexahydro-2,5,6-trioxopyrazino[1,2,3-de]quinoxaline-3-propionic Acid **1e**

The title compound was obtained by hydrolysis of **1d** (0.66 mmol) as described to prepare **1b** [10].

Table 2. Physical and ^{1}H NMR data of the intermediates.

Comp	Mp(°C)	Solv[a]	Yield(%)	^{1}H NMR (DMSO-d_6)
5	oil		78	5.05 (d, 1H, CH, *J* = 6.0 Hz), 6.93 (t, 1H, ar, *J* = 8.2 Hz), 7.11–7.29 (m, 5H, ar), 8.23 (d, 2H, ar, *J* = 8.2 Hz), 9.57 (d, 1H, NH, *J* = 6.0 Hz).
6	oil		80	1.95–2.03 (m, 2H, CH_2), 2.25–2.32 (m, 2H, CH_2), 3.77–3.88 (m, 1H, CH), 7.07 (t, 1H, ar, *J* = 8.2 Hz), 7.99 (d, 1H, NH, *J* = 9.4 Hz), 8.30 (d, 2H, ar, *J* = 8.2 Hz), 12.45 (br s, exchangeble protons).
7c	oil		75	1.10 (t, 3H, CH_3, *J* = 7.1 Hz), 4.15 (q, 2H, CH_2, *J* = 7.1 Hz), 5.14 (d, 1H, CH, *J* = 6.4 Hz), 6.98 (t, 1H, ar, *J* = 8.2 Hz), 7.16–7.33 (m, 5H, ar), 8.27 (d, 2H, ar, *J* = 8.2 Hz), 9.41 (d, 1H, NH, *J* = 6.4 Hz).
7d	oil		87	1.09–1.20 (m, 6H, 2 CH_3), 1.99–2.10 (m, 2H, CH_2), 2.38–2.45 (m, 2H, CH_2), 3.78–3.89 (m, 1H, CH), 3.97–4.14 (m, 4H, 2CH_2 esters), 7.11 (t, 1H, ar, *J* = 8.2 Hz), 7.73 (d, 1H, NH, *J* = 9.8 Hz), 8.29 (d, 2H, ar, *J* = 8.2 Hz).
8c	210–213	A	50	4.78–4.90 (m, 3H, H-3 + NH_2), 5.65 (s, 1H, NH), 6.10 (d, 1H, ar, *J* = 7.3 Hz), 6.26 (d, 1H, ar, *J* = 7.3 Hz), 6.43 (t, 1H, ar, *J* = 7.3 Hz), 7.27 (s, 5H, ar), 10.21 (s, 1H, NH).
8d	87–89	A	60	1.18 (t, 3H, CH_3, *J* = 7.0 Hz), 1.76–1.93 (m, 2H, CH_2), 2.43–2.47 (m, 2H, CH_2), 3.67–3.70 (m, 1H, H-3), 4.06 (q, 2H, CH_2, *J* = 7.0 Hz), 4.76 (s, 2H, NH_2), 4.99 (d, 1H, NH, *J* = 2.4 Hz), 6.11 (d, 1H, ar, *J* = 7.6 Hz), 6.24 (d, 1H, ar, *J* = 7.6 Hz), 6.43 (t, 1H, ar, *J* = 7.6 Hz), 10.07 (s, 1H, NH).
9	246–247	B	75	2.85 (t, 2H, CH_2, *J* = 7.1 Hz), 3.18 (t, 2H, CH_2, *J* = 7.1 Hz), 7.35 (t, 1H, ar, *J* = 8.1 Hz), 8.03 (d, 1H, ar, *J* = 8.1 Hz), 8.07 (d, 1H, ar, *J* = 8.1 Hz), 12.30 (br s, 1H, NH or OH), 13.00 (br s, 1H, OH or NH).
10a	172–174	C	90	1.17 (t, 3H, CH_3, *J* = 7.0 Hz), 2.92 (t, 2H, CH_2, *J* = 7.1 Hz), 3.22 (t, 2H, CH_2, *J* = 7.1 Hz), 4.07 (q, 2H, CH_2, *J* = 7.0 Hz), 7.36 (t, 1H, ar, *J* = 8.1 Hz), 8.02 (d, 1H, ar, *J* = 8.1 Hz), 8.08 (d, 1H, ar, *J* = 8.1 Hz), 13.07 (s, 1H, NH).
10b	222–224	D	70	2.96 (t, 2H, CH_2, *J* = 7.7 Hz), 3.26 (t, 2H, CH_2, *J* = 7.7 Hz), 7.05 (t, 1H, ar, *J* = 7.7 Hz), 7.24–7.38 (m, 3H, ar), 7.59 (d, 2H, ar, *J* = 8.3 Hz), 7.99–8.10 (m, 2H, ar), 10.07 (s, 1H, NH), 13.08 (s, 1H, NH).
10c	174–176	D	58	3.00 (t, 2H, CH_2, *J* = 7.0 Hz), 3.25 (t, 2H, CH_2, *J* = 7.0 Hz), 5.10 (s, 2H, CH_2), 7.29 (s, 5H, ar), 7.37 (t, 1H, ar, *J* = 8.1 Hz), 8.02 (d, 1H, ar, *J* = 8.1 Hz), 8.08 (d, 1H, ar, *J* = 8.1 Hz), 13.15 (br s, 1H, NH).
11a	83–85	E	75	1.18 (t, 3H, CH_3, *J* = 7.1 Hz), 2.84 (t, 2H, CH_2, *J* = 6.9 Hz), 2.96 (t, 2H, CH_2, *J* = 6.9 Hz), 4.05 (q, 2H, CH_2, *J* = 7.1 Hz), 5.04 (s, 2H, NH_2), 6.29 (d, 1H, ar, *J* = 7.7 Hz), 6.60 (d, 1H, ar, *J* = 7.7 Hz), 6.81 (t, 1H, ar, *J* = 7.7 Hz), 11.89 (s, 1H, NH).
11b	192–194	F	88	2.89 (t, 2H, CH_2, *J* = 7.1 Hz), 3.10 (t, 2H, CH_2, *J* = 7.1 Hz), 5.15 (br s, 2H, NH_2), 6.33 (d, 1H, ar, *J* = 7.5 Hz), 6.66 (d, 1H, ar, *J* = 7.7 Hz), 6.82 (t, 1H, ar, *J* = 7.5 Hz), 7.03 (t, 1H, ar, *J* = 7.5 Hz), 7.30 (t, 2H, ar, *J* = 7.7 Hz), 7.61 (d, 2H, ar, *J* = 7.7 Hz), 10.08 (s, 1H, NH).
11c	107–108	E	75	2.94 (t, 2H, CH_2, *J* = 6.0 Hz), 3.03 (t, 2H, CH_2, *J* = 6.0 Hz), 5.04 (s, 2H, NH_2), 5.12 (s, 2H, CH_2), 6.28 (d, 1H, ar, *J* = 7.7 Hz), 6.60 (d, 1H, ar, *J* = 7.7 Hz), 6.82 (t, 1H, ar, *J* = 7.7 Hz), 7.32 (s, 5H, ar), 11.91 (s, 1H, NH).

[a]Purification solvents: A = Benzene. B = Glacial acetic acid. C = Ethyl acetate. D = Ethanol. E = Diethyl ether. F = Acetonitrile.

Table 3. Physical and ^{1}H NMR data of the final tricyclic compounds.

Comp	Mp(°C)	Solv[a]	Yield(%)	^{1}H NMR (DMSO-d_6)
1c	268–272	A	30	5.95 (s, 1H, H-3), 6.78 (d, 1H, ar, *J* = 8.0 Hz), 6.88 (d, 1H, ar, *J* = 8.0 Hz), 7.12–7.34 (m, 6H, ar), 11.14 (s, 1H, NH), 12.25 (s, 1H, NH).
1d	248–251	B	45	1.07 (t, 3H, CH_3, *J* = 7.2 Hz), 2.04–2.36 (m, 4H, $2CH_2$), 3.86–3.91 (m, 2H, CH_2 esters), 5.03–5.08 (m, 1H, H-3), 6.69 (d, 1H, ar, *J* = 7.7 Hz), 6.79 (d, 1H, ar, *J* = 7.7 Hz), 7.08 (t, 1H, ar, *J* = 7.7 Hz), 11.09 (s, 1H, NH), 12.07 (s, 1H, NH).
1e	> 300	A	80	2.02–2.27 (m, 4H, $2CH_2$), 5.02–5.07 (m, 1H, H-3), 6.69 (d, 1H, ar, *J* = 8.2 Hz), 6.78 (d, 1H, ar, *J* = 8.2 Hz), 7.07 (t, 1H, ar, *J* = 8.2 Hz), 11.10 (s, 1H, NH), 12.15 (br s, 2H, NH + OH).
1f	> 300	C	60	3.09–3.32 (m, 2H, CH_2), 5.15–5.21 (m, 1H, H-3), 6.67–6.77 (m, 2H, ar), 6.98–7.09 (m, 2H, ar), 7.17–7.35 (m, 4H, ar), 10.01 (s, 1H, NH), 11.00 (s, 1H, NH), 12.11 (s, 1H, NH).
1g	265–267	D	50	2.15–2.32 (m, 4H, $2CH_2$), 5.03–5.15 (m, 1H, H-3), 6.67–6.75 (m, 2H, ar), 6.98–7.08 (m, 2H, ar), 7.19–7.26 (m, 2H, ar), 7.42–7.46 (m, 2H, ar), 9.77 (s, 1H, NH), 11.06 (s, 1H, NH), 12.15 (s, 1H, NH).
2a	246–248	E	75	1.01 (t, 3H, CH_3, *J* = 7.0 Hz), 3.21 (dd, 1H, CH_2, *J* = 17.2, 3.0 Hz), 3.48 (dd, 1H, CH_2, *J* = 17.2, 4.6 Hz), 3.94 (q, 2H, CH_2, *J* = 7.0 Hz), 5.02–5.05 (m, 1H, H-4), 6.48 (d, 1H, ar, *J* = 7.7 Hz), 6.62 (d, 1H, ar, *J* = 7.7 Hz), 6.86 (t, 1H, ar, *J* = 7.7 Hz), 10.79 (s, 1H, NH), 10.85 (s, 1H, NH).
2b	279–282	D	80	3.15 (dd, 1H, CH_2, *J* = 17.5, 3.0 Hz), 3.47 (dd, 1H, CH_2, *J* = 17.5, 4.2 Hz), 4.95–4.98 (m, 1H, H-4), 6.46 (d, 1H, ar, *J* = 7.9 Hz), 6.61 (d, 1H, ar, *J* = 7.9 Hz), 6.85 (t, 1H, ar, *J* = 7.9 Hz), 10.72 (s, 1H, NH), 10.84 (s, 1H, NH), 12.40 (s, 1H, OH).
2c	> 300	D	65	5.77 (s, 1H, H-4), 6.57 (d, 1H, ar, *J* = 7.8 Hz), 6.71 (d, 1H, ar, *J* = 7.8 Hz), 6.94 (t, 1H, ar, *J* = 7.8 Hz), 7.15–7.19 (m, 2H, ar), 7.33–7.38 (m, 3H, ar), 10.90 (s, 1H, NH), 10.94 (s, 1H, NH).
2d	212–214	E	70	1.07 (t, 3H, CH_3, *J* = 7.1 Hz), 2.19–2.36 (m, 4H, $2CH_2$), 3.83–3.96 (m, 2H, CH_2 esters), 4.89–4.92 (m, 1H, H-4), 6.47 (d, 1H, ar, *J* = 7.9 Hz), 6.62 (d, 1H, ar, *J* = 7.9 Hz), 6.86 (t, 1H, ar, *J* = 7.9 Hz), 10.82 (s, 1H, NH), 10.90 (s, 1H, NH).
2e	> 300	A	75	2.11–2.46 (m, 4H, $2CH_2$), 4.88–4.91 (m, 1H, H-4), 6.47 (d, 1H, ar, *J* = 7.8 Hz), 6.62 (d, 1H, ar, *J* = 7.8 Hz), 6.86 (t, 1H, ar, *J* = 7.8 Hz), 10.82 (s, 1H, NH), 10.94 (s, 1H, NH), 12.15 (s, 1H, OH).
2f	> 300	D	60	3.26 (dd, 1H, CH_2, *J* = 16.5, 2.9 Hz), 3.69 (dd, 1H, CH_2, *J* = 16.5, 4.0 Hz), 4.96–5.09 (m, 1H, H-4), 6.50 (d, 1H, ar, *J* = 7.9 Hz), 6.61 (d, 1H, ar, *J* = 7.9 Hz), 6.83–7.03 (m, 2H, ar), 7.22 (t, 2H, ar, *J* = 7.9 Hz), 7.41 (d, 2H, ar, *J* = 7.9 Hz), 10.09 (s, 1H, NH), 10.70 (s, 1H, NH), 10.77 (s, 1H, NH).
2g	287–289	D	70	2.10–2.47 (m, 4H, $2CH_2$), 4.91–4.99 (m, 1H, H-4), 6.48 (d, 1H, ar, *J* = 7.7 Hz), 6.64 (d, 1H, ar, *J* = 7.7 Hz), 6.84 (t, 1H, ar, *J* = 7.7 Hz), 7.01 (m, 1H, ar), 7.26 (t, 2H, ar, *J* = 7.7 Hz), 7.51 (d, 2H, ar, *J* = 7.7 Hz), 9.87 (s, 1H, NH), 10.86 (s, 1H, NH), 10.92 (s, 1H, NH).
3a	206–207	F	35	1.21 (t, 3H, CH_3, *J* = 7.1 Hz), 2.94 (t, 2H, CH_2, *J* = 7.0 Hz), 3.49 (t, 2H, CH_2, *J* = 7.0 Hz), 4.11 (q, 2H, CH_2, *J* = 7.1 Hz), 7.01 (d, 1H, ar, *J* = 7.6 Hz), 7.32 (d, 1H, ar, *J* = 7.6 Hz), 7.42 (t, 1H, ar, *J* = 7.6 Hz), 12.02 (s, 1H, NH).
3b	247–250	B	38	2.95–3.03 (m, 2H, CH_2), 3.49–3.58 (m, 2H, CH_2), 6.99–7.07 (m, 2H, ar), 7.22–7.44 (m, 4H, ar), 7.57–7.64 (m, 2H, ar), 10.08 (s, 1H, NH), 12.03 (s, 1H, NH).
3c	212–213	F	30	3.03 (t, 2H, CH_2, *J* = 6.9 Hz), 3.52 (t, 2H, CH_2, *J* = 6.9 Hz), 5.12 (s, 2H, CH_2), 7.01 (d, 1H, ar, *J* = 7.5 Hz), 7.30–7.46 (m, 7H, ar), 12.02 (s, 1H, NH).
3d	233–234	F	60	2.86 (t, 2H, CH_2, *J* = 7.1 Hz), 3.44 (t, 2H, CH_2, *J* = 7.1 Hz), 7.01 (d, 1H, ar, *J* = 7.5 Hz), 7.29–7.45 (m, 2H, ar), 11.99 (s, 1H, NH or OH), 12.30 (brs, 1H, OH or NH).

[a]Purification solvents: A = Dimethylformamide/water. B = Nitromethane. C = Dimethylformamide. D = Glacial acetic acid. E = Ethanol. F = Ethyl acetate.

(±)1,2,3,5,6,7-Hexahydro-2,5,6-trioxopyrazino[1,2,3-de]quinoxaline-3-N-phenylacetamide **1f** *and (±) 1,2,3,5,6,7-Hexahydro-2,5,6-trioxopyrazino[1,2,3-de]quinoxaline-3-N-phenylpropionamide* **1g**

Equimolar amount (0.68 mmol) of aniline, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride and 1-hydroxy-1*H*-benzotriazole were added to a solution of acid **1b, e** (0.62 mmol) in anhydrous dimethylformamide (DMF) (3 mL). The mixture was stirred at room temperature for 20 h. Addition of HCl (0.1 M, 10 mL) yielded a solid which was collected and washed with H_2O.

(±) 2,4,5,6-Tetrahydro-2,5-dioxo-1H-imidazo[1,5,4-de]quinoxalin-4-acetic Acid **2b** *and (±) 2,4,5,6-Tetrahydro-2,5-dioxo-1H-imidazo[1,5,4-de]quinoxaline-4-propionic Acid* **2e**

The title compounds were obtained by hydrolysis of the corresponding esters **2a, d** (2.96 mmol) as described for the synthesis of **1b** [10].

(±) 2,4,5,6-Tetrahydro-2,5-dioxo-1H-imidazo[1,5,4-de]quinoxalin-4-N-phenylacetamide **2f** *and (±) 2,4,5,6-Tetrahydro-2,5-dioxo-1H-imidazo[1,5,4-de]quinoxaline-4-N-phenylpropionamide* **2g**

The title compounds were obtained from the acids **2b, e** (1.46 mmol) as described above to prepare **1f–g**.

4-Nitrobenzimidazole-2-propionic Acid **9**

An excess of succinic anhydride (39.2 mmol) was added to a suspension of 3-nitrophenylene-1,2-diamine (19.6 mmol) in xylene (100 mL). The mixture was refluxed for 6 h. The crude acid **9**, that resulted upon cooling, was collected and purified as follows: the acid **9** was suspended in H_2O (35 mL) and the suspension was slightly alkalinized (pH ≈ 8) with NaOH (10%). The solid was filtered off and the clear solution was acidified with glacial acetic acid to yield a solid which was collected and washed with H_2O.

Ethyl 4-Nitrobenzimidazole-2-propionate **10a**

Conc H_2SO_4 (0.89 mL) was added to a solution of **9** (12.4 mmol) in absolute EtOH (130 mL). The solution was refluxed for 3 h. Elimination of the solvent at reduced pressure yielded a residue which was treated with H_2O (60 mL). The mixture was alkalinized with $NaHCO_3$ (saturated solution) to produce a solid which was collected and washed with H_2O.

4-Nitrobenzimidazole-2-N-phenylpropionamide **10b**

A solution of **9** (2.5 mmol) in anhydrous DMF (5 mL) was added dropwise and under stirring to a cooled (0 °C) suspension of 1-hydroxy-1*H*-benzotriazole (5.1 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (5.1 mmol) and aniline (3 mmol) in anhydrous DMF. The mixture was stirred at room temperature for 12 h. Dilution with H_2O (15 mL) and alkalization with $NaHCO_3$ (saturated solution) yielded a solid which was collected and washed with H_2O.

Benzyl 4-Nitrobenzimidazole-2-propionate **10c**

K_2CO_3 (0.48 g) was added to a stirred solution of **9** (4.59 mmol) in anhydrous DMF (60 mL). The mixture was stirred at room temperature for 4 h. Benzyl chloride (5.5 mmol) was added to the mixture which was stirred, still at room temperature, for another 24 h. The mixture was then concentrated (30 mL) at reduced pressure and at 100 °C and diluted with H_2O (70 mL) to yield a solid which was collected, washed with H_2O and purified by column chromatography, eluting system $CHCl_3$/ethyl acetate (9:1). Evaporation of the central eluates gave a residue which was washed with Et_2O.

Ethyl 4-Aminobenzimidazole-2-propionate **11a**

Conc HCl (0.25 mL) and Pd/C (10%, 0.4 g) were added to a solution of **10a** (1.5 mmol) in absolute EtOH (40 mL). The mixture was hydrogenated in a Parr apparatus at 40 psi for 2 h. Elimination of the catalyst and evaporation at reduced pressure of the solvent yielded an oily residue which was dissolved in H_2O (15 mL). The solution was alkalinized with $NaHCO_3$ (saturated solution) and extracted with ethyl acetate (3 × 30 mL). The organic layers were washed with H_2O (3 × 20 mL) and dried (Na_2SO_4). Evaporation of the solvent at reduced pressure yielded an oily residue which became solid upon treatment with petroleum ether (40–60 °C) .

4-Aminobenzimidazole-2-N-phenylpropionamide **11b**

Pd/C (10%, 0.1 g) was added to a hot solution of **10b** (1.2 mmol) in absolute EtOH (100 mL). The mixture was hydrogenated in a Parr apparatus at 40 psi for 3 h. Elimination of the catalyst and evaporation of the solvent at reduced pressure yielded a residue which became solid with Et_2O.

Benzyl 4-Aminobenzimidazole-2-propionate **11c**.

A solution of **10c** (1.63 mmol) in acetone (50 mL) was slowly (1 h) added to a stirred and cooled (0 °C) solution of $TiCl_3$ (20%, in H_2O, 16.3 mmol) in H_2O (5 mL) and acetone (5 mL). The mixture was stirred and cooled for 30 min. The acetone was evaporated at reduced pressure and the aqueous mixture was added with H_2O (70 mL) and ethyl acetate (150 mL), and then alkalinized with $NaHCO_3$ (saturated solution). The mixture was stirred for 30 min. The solid was filtered off and the two layers were separated. Evaporation at reduced pressure of the dried (Na_2SO_4) organic solvent yielded an oily residue which became solid upon treatment with Et_2O.

Ethyl 5,6-Dihydro-4,5-dioxo-4H-imidazo[1,5,4-de]quinoxaline-2-propionate **3a**, *5,6-Dihydro-4,5-dioxo-4H-imidazo[1,5,4-de]quinoxaline-2-N-phenylpropionamide* **3b**, *and Benzyl 5,6-Dihydro-4,5-dioxo-4H-imidazo[1,5,4-de]quinoxaline-2-propionate* **3c**

A solution of **11a–c** (2.14 mmol) and triethylamine (4.28 mmol) in THF (20 mL) was slowly (3 h) added under nitrogen atmosphere to a stirred and cooled (0 °C) solution of oxalyl chloride (2.35 mmol) in THF (20 mL). The mixture was stirred and cooled for 30 min. Dilution with H_2O (70 mL) yielded compounds **3a–b** as a milky suspension which was extracted with ethyl acetate (3 × 40 mL). The organic layers were washed with H_2O (2 × 50 mL) and dried (Na_2SO_4). Evaporation of the solvent at reduced pressure afforded a residue which was treated with Et_2O and collected. In the case of **3c** the dilution with H_2O gave a solid which was collected, washed with H_2O, and suspended in ethyl acetate (40 mL). The solid was filtered off and the solution was concentrated (10 mL) to yield crude **3c**.

5,6-Dihydro-4,5-dioxo-4H-imidazo[1,5,4-de]quinoxaline-2-propionic Acid **3d**

Pd/C (10%, 0.1 g) was added to a solution of **3c** (0.51 mmol) in ethyl acetate (200 mL). The mixture was hydrogenated in a Parr apparatus at 35 psi for 2 h. Elimination of the catalyst and evaporation at reduced pressure of the solvent yielded a residue which was washed with Et_2O.

Biochemistry

Synaptic Membrane Preparation

Crude synaptic membranes were prepared from the cerebral cortex plus hippocampus of male Sprague-Dawley rats (170–250 g). The tissue was homogenized 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 1,000 × *g* for 10 min and the resulting supernatant further centrifuged at 20,000 × *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 8,000 × *g* for 20 min. The supernatant and the soft upper layer of the pellet were collected together and centrifuged at 48,000 × *g* for 20 min. The membranes were resuspended once more in distilled water, centrifuged and frozen at –80 °C.

[^{3}H]Glycine Binding

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 48,000 × *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) 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.

[^{3}H]DL-AMPA Binding

[^{3}H]DL-AMPA binding assays were carried out basically following the method of Nielsen et al.[12] Briefly, frozen membranes were resuspended in cold 30 mM Tris-HCl buffer, pH 7.1, containing 2.5 mM $CaCl_2$ and centrifuged at 48,000 × *g* for 10 min. After an additional washing step, the pellet was resuspended (0.5–0.6 mg protein/mL) in the original buffer containing 2.5 mM $CaCl_2$ and 100 mM KSCN and used for binding assays. Aliquots of 0.5 mL membrane suspensions in triplicate were incubated for 30 min at 0–2 °C with 5 nM [^{3}H]DL-AMPA (40 Ci/mmol) in the absence or presence of scalar concentrations of the test compound. Non-specific binding was assessed in the presence of 10 µM 6-nitro-7-sulfamoylbenzo[*f*]quinoxaline-2,3-dione (NBQX). Bound radioactivity was separated by filtration through Whatman GF/C glass fibre discs followed by washing with 3 × 5 mL ice-cold buffer.

Sample Preparation and Result Calculation

A stock 1 mM solution of the test compound was prepared in 50% 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[13] and, in the case of tritiated glycine and AMPA binding, converted to K_i values by application of the Cheng-Prusoff equation.[14]

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