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N-PHOSPHONOALKYL-5-AMINOMETHYLQUINOXALINE-2,3-DIONES: *IN VIVO* ACTIVE AMPA AND NMDA(GLYCINE) ANTAGONISTS.

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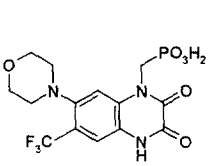
Abstract: N-Substituted 5-aminomethylquinoxalinediones containing carboxy or phosphonic acids yield potent and selective AMPA and/or NMDA (glycine-binding site) antagonists. Phosphonic acid derivatives are particularly water-soluble and display potent anticonvulsant effects in the electroshock-induced convulsion assay in mice.

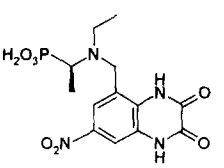
L-Glutamate is the major excitatory neurotransmitter in the mammalian nervous system. Excessive activation of a number of glutamate receptors has been linked to various pathological situations, and compounds capable of interacting with glutamatergic neurotransmission are potential candidates for new therapies of e.g. pain[1], amyotrophic lateral sclerosis[2], anxiety[3], epilepsy[4], Parkinson's disease[5] and cerebral ischemia[6].

It has been well documented that quinoxaline-2,3-dione derivatives may act as antagonists at the AMPA/kainate[7] and NMDA[8] subtypes of ionotropic glutamate receptors. Representative examples are the AMPA antagonist MPQX[7e], which is in clinical development for the treatment of stroke, or the NMDA glycine-site antagonist SM-18400[8a].

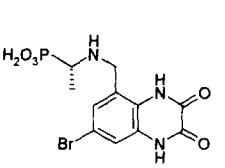
In this article, we present 5-aminomethylquinoxaline-2,3-diones derivatives showing high affinities for AMPA receptors and/or for the glycine-binding site of NMDA receptors. These compounds display markedly improved anticonvulsant effects in the electroshock-induced convulsion model in mice (ESM)[9], in comparison to previously published analogues[10].



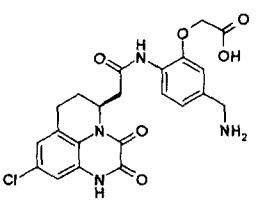
MPQX



1f



2b



SM-18400

	MPQX	1f	2b	SM-18400
AMPA[a]	105 nM	80 nM	1.2 μM	no affinity[8a]
MDL[b]	3.3 μM	13%	6 nM	0.5 nM[8a]
ESM[c]:	5 mg/kg	7 mg/kg	12 mg/kg	n.a.

a) IC_{50} in the [^{3}H]AMPA binding assay[13a]; b) IC_{50} or % inhibition at 1μM in the [^{3}H]-(Z)-2-carboxy-4,6-dichloroindole-3-(2'-phenyl-2'-carboxy)-ene ([^{3}H]MDL-105519) binding assay[13b]; c) ED_{50} [mg/kg] 30 min. after i.p. administration; n.a. not available.

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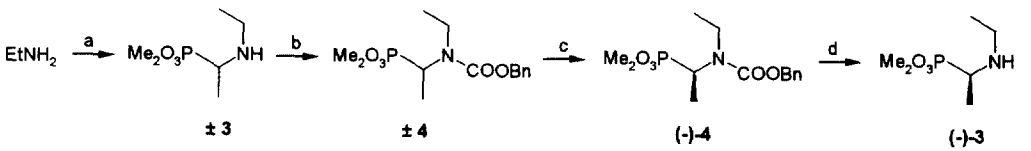
Chemistry

Racemic N-ethyl-phosphoalanine dimethylester (± **3**, Scheme 1) was obtained directly from ethylamine, then protected as its benzyl carbamate ± **4**. The latter was resolved on a 500g-scale using simulated moving-bed chromatography[11], and hydrogenated to the unstable optically active phosphoalanine **3**[12].

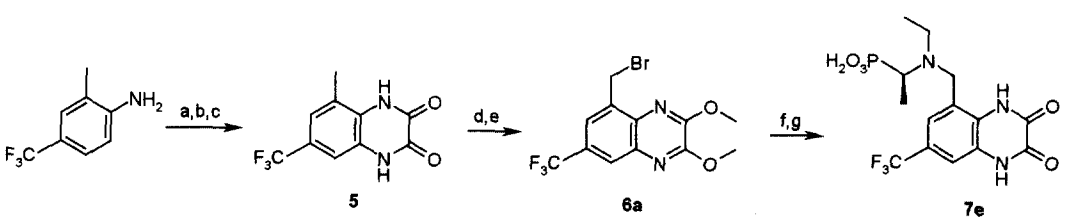
Scheme 1



Reagents and conditions: a) i. MeCHO, ii. $P(OMe)_3$, 77% after distillation; b) BnOOC-Cl, Et_3N, 92%; c) resolution by simulated moving-bed chromatography on Chiralcel OJ, e.e. = 99%, yield = 35%; d) H_2, 5% Pd/C, MeOH, 99%.

N-Phosphonoalkyl-5-aminomethylquinoxaline-2,3-diones can be prepared via alkylation (e.g. **7e**, Scheme 2), or reductive amination (Scheme 3). For instance, treatment of 2-methyl-4-trifluoro-methylaniline with ethyl oxalyl chloride, followed by nitration and cyclization under reductive conditions ($TiCl_3$, aq. HCl) afforded **5** (Scheme 2). The dione was then protected as a dimethylether, and the benzylic position brominated with NBS to give **6a**. Alkylation of freshly prepared aminophosphonate **3** with bromide **6a**, and acid hydrolysis, yielded **7e**.

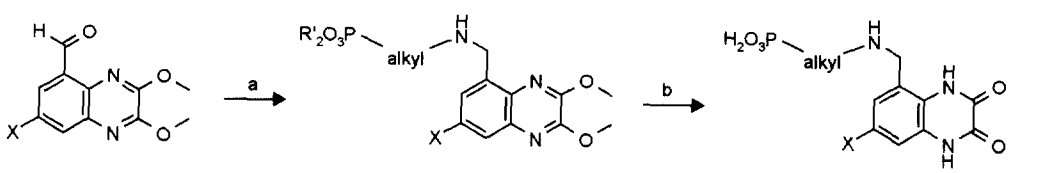
Scheme 2



Reagents and conditions: a) AcOEt, Et_3N, EtOOC-COCl, 3°C, 89%; b) H_2SO_4, KNO_3, 0°C, 88%; c) $TiCl_3$, aq. HCl, acetone, 0°C, 94%; d) i. $POCl_3$, PCl_5, reflux, 85%; ii. MeOH, MeONa, reflux, 75%; e) NBS, AIBN, C_6H_6, reflux, 2h, 67%; f) **3**, DMF, $NaHCO_3$, 61%; g) conc. HCl, 60°C, 82%.

Alternatively, aldehydes **10a-c**[10b] were coupled to aminophosphonates under reductive amination conditions, and deprotected by treatment with concentrated HCl:

Scheme 3

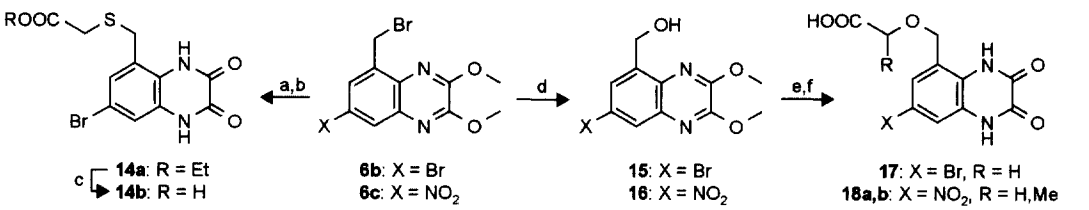


Reagents and conditions: a) R'_2O_3P-alkyl-NH_2, CH_2Cl_2, $MgSO_4$, RT, 18h, then $NaBH_3CN$, MeOH, 4h, 52-89%; b) conc. HCl, RT to reflux, 62-100%.

7-Bromo and 7-nitro amino acid derivatives **8b-j** and **9a-d** (Table 1) were obtained from the corresponding 5-bromomethyl intermediates **6b,c**[10a], as exemplified in Scheme 2. Yields were 54-95% for the alkylation, and 65-98% for the deprotection step.

Thioether **14b** was prepared by reaction of **6b** with ethyl 2-thioacetate under basic conditions, followed by hydrolysis (Scheme 4). Ethers **17** and **18a,b** were obtained under phase-transfer conditions from the hydroxymethyl derivatives **15** or **16**.

Scheme 4



Reagents and conditions: a) EtOH, EtONa, EtOOC-CH_2-SH, THF, RT, 18h, ; b) 33% HBr in AcOH, 130°C; c) LiOH, THF, H_2O, 46% from **6b**; d) H_2O, dioxane, $CaCO_3$, reflux, 24h; e) $Bu_4N^+HSO_4^-$, CH_2Cl_2, 40% aq. NaOH, *t*-BuOOC-CH(R)Br, RT, 18h; f) AcOH, 2N HCl, reflux, 2h, 21-28% from **6c**.

Al compounds were characterized by ^{1}H-NMR(250 MHz) and mass spectroscopy[14].

Results and discussion (Table 1)

Amino acid derivatives. Compound **8b**, formally derived from the selective AMPA antagonist **8a**[10a] by opening the piperidine ring, showed a weaker potency in the AMPA-binding test. Shortening the linker betweeen the carboxylate group and the nitrogen atom (**8c,d**) improved the affinity for AMPA receptors, which was shown to decrease again with larger substituents on the amino acid side-chain (**8d-h**). The stereochemistry[15] of the alanine derivatives had little influence on the binding affinity (**8e,f**), but N-methylation improved the selectivity for AMPA receptors (**8i**). Finally, esterification decreased the potency at both receptor subtypes (**8j**). These amino acid derivatives are soluble in phosphate buffer (e.g. **8e**: 1.85 g/L at 25°C, pH of the saturated solution = 7.4). Replacement of the carboxylate group of the alanine derivative with a tetrazole ring[16] had little effect on *in vitro* potency (IC_{50} value at AMPA receptors : 430 nM), but the *in vivo* activity disappeared.

Since **8d**, and to a lesser extent **8e** and **8f**, also display some affinity for the glycine-binding site of NMDA receptors, we replaced the 7-nitro group by a bromine atom, to see whether we could also improve the affinity for the glycine-binding site of NMDA receptors. Among the resulting compounds (**9a-d**), the most potent and selective glycine antagonist is **9a**. The D-alanine derivative **9b** has a similar potency, whereas the L-enantiomer **9c** is markedly weaker.

The amino acid derivatives were evaluated for *in vivo* activity in the maximal electroshock model in mice, and several of these compounds were active after intraperitoneal administration and thirty minutes pretreatment time. However, even the most potent derivative **8e** (ED_{50} = 19 mg/kg) showed no remaining activity at 50 mg/kg i.p.

after one hour, a result indicative of a short duration of action. This compound was also inactive after oral administration (100 mg/kg, 1h).

Thio- and hydroxy acid derivatives. Affinities for the glycine-binding site of NMDA receptors were little affected by replacement of the nitrogen with a sulfur (**14b**) or an oxygen atom (**17**). In contrast, the affinity at AMPA receptors was more sensitive to the replacement of the nitrogen by an oxygen atom, and **18a,b** are much less potent than their nitrogen analogs **8d,e**. These compounds were markedly less soluble in water than the corresponding amino acid derivatives.

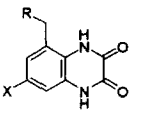
Amino phosphonic acid derivatives. Phosphoglycine derivative **1a** binds to AMPA receptors and to the glycine-binding site of NMDA receptors, but N-ethylation increases its preference for AMPA receptors (**1b**). The D-phosphoalanine derivative **1c** is a potent anticonvulsants (ED_{50} = 3 mg/kg), clearly prefering the glycine-binding site of NMDA receptors. Its N-ethylated derivative (**1e**) is weak *in vivo*, in contrast to the N-ethylated L-enantiomer[13] **1f**, which shows a good activity in the ESM after i.p. administration and is selective for AMPA receptors. This compound has a longer duration of action than its amino acid analogue, but is inactive after oral administration (100 mg/kg, 1h). Replacement of the N-ethyl group by N-acetyl decreased the affinity for AMPA receptors (**1g**). The β-phosphoalanine derivative **1h** is less potent than its shorter analogue **1a**, but is more selective. Introduction of a methyl group on its β position led to improved *in vivo* effects (**1i,j**). The D- and L-enantiomers show practically no difference in affinity for AMPA receptors and in their anticonvulsant activity.

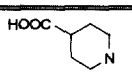
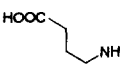
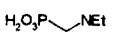
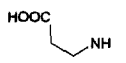
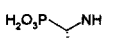
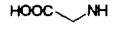
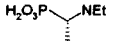
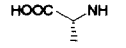
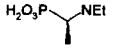
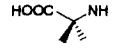
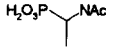
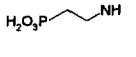
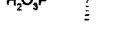
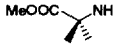
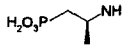
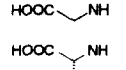
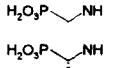
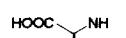
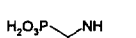
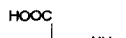
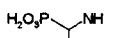
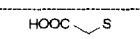
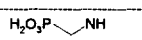
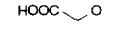
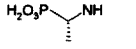
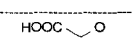
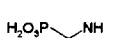
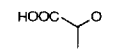
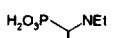
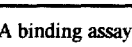
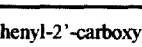
In **2a-d**, the 7-nitro group was replaced by a bromine atom. The D-phosphoalanine derivative **2b** was shown to be highly potent with excellent selectivity for the glycine-binding site of NMDA receptors. In the MES test, this compound displayed a very good anticonvulsant effect after intraperitoneal administration, with a duration of action similar to **1f**: after two hours, its ED_{50} is 17,7 mg/kg (i.p.). Like the other derivatives in this series, it shows no activity after oral administration (100 mg/kg, 1h).

Replacement of the 7-bromo by a 7-CF_3 group (**7a-d**) caused some decrease in selectivity for the glycine-binding site of NMDA receptors. In the phosphoalanine series, the D-enantiomer **7b** proved very potent at the glycine-binding site of NMDA receptors, whereas its enantiomer **7c** remained only moderately active. As expected, N-ethylation produced an increase in selectivity and affinity for AMPA receptors (**7d**).

Phosphonoalkyl derivatives are markedly more soluble than their amino acid counterparts (e.g. **1f**: 16.7 g/L at 22°C, pH of the saturated solution = 6.76). The log P values of these quinoxaline-2,3-diones are lower than those usually expected to allow brain penetration (e.g. **8e**: log P = -1.93, **1f**: log P = -3.5). However, their excellent *in vivo* activity might be explained by the involvement of an active transport system, as was suggested for similar compounds[7e and ref. cited therein].

Table 1: Structures, *in vitro* affinities and *in vivo* potencies of the 5-amino-quinoxaline-2,3-diones



	Carboxylic Acid Derivatives						Phosphonic Acid Derivatives				
	X	R	AMPA[a,f]	MDL[b,f]	ESM[c]		X	R	AMPA[a,f]	MDL[b,f]	ESM[c]
8a	NO_2		0.07	3.9	44	**1a**	NO_2		0.29	1.0	7
8b	NO_2		0.61	9%	n.t.	**1b**[d]	NO_2		0.12	24%	18
8c[d]	NO_2		0.38	19%	35	**1c**	NO_2		0.17	0.032	3
8d[d]	NO_2		0.16	0.76	32 (15 min)	**1d**	NO_2		0.38	51%	8
8e[d]	NO_2		0.31	1.7	19, 0% (1h)	**1e**	NO_2		0.31	25%	26
8f[d]	NO_2		0.38	1.3	0%	**1f**	NO_2		0.08	13%	7, 18 (2h)
8g[e]	NO_2		0.29	-9%[g]	0%	**1g**	NO_2		1.3	31%	n.t.
8h[d]	NO_2		1.2	34%	0%	**1h**[d]	NO_2		0.64	8%	18
8i[d]	NO_2		0.34	22%	0%	**1i**[e]	NO_2		0.20	6%	9
8j[d]	NO_2		1.9	-3%[g]	n.t.	**1j**[e]	NO_2		0.20	15%	10
9a[d]	Br		4.7	0.04	0%	**2a**	Br		2.4	0.1	0%
9b	Br		4.5	0.12	n.t.	**2b**	Br		3	0.006	12, 18 (2h)
9c[d]	Br		4.3	2.2	n.t.	**2c**	Br		1.4	0.37	43
9d	Br		3.8	37%	n.t.	**2d**	Br		16%	0.045	20%
14b	Br		22%	0.12	0% (1h)	**7a**	CF_3		2.0	0.3	30
17	Br		4%	0.11	0% (1h)	**7b**[e]	CF_3		1.2	0.006	8
18a	NO_2		2.0	0.31	n.t.	**7c**[e]	CF_3		0.54	0.59	20
18b	NO_2		1.3	0.42	n.t.	**7d**	CF_3		0.068	4	10

a: [^{3}H]-AMPA binding assay[13a]; b: [^{3}H]-(Z)-2-carboxy-4,6-dichloroindole-3-(2'-phenyl-2'-carboxy)-ene ([^{3}H]MDL-105519) binding assay[13b]; c) ED_{50} [mg/kg] or % protection at 50 mg/kg, 30 min. (i.p. injection, unless otherwise stated), n = 5 per dose; d) HBr salt; e) HCl salt; f) average of at least two independent experiments run in triplicate: IC_{50} ± SEM in μM (from 6 or 12 concentrations of each compound), or % inhibition at 1 μM; g) negative data within the normal variation range of results for inactive compounds.

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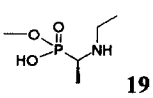
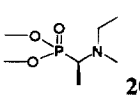
12. (-)-**3** must be converted rapidly, as it decomposes upon standing to **19** and **20**. The stereochemistry of (-)-**3** was confirmed by comparison with the ethylation product of dimethyl-(L)-phosphoalanine.



19



20

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14. E.g. **2b** (mw = 378.12): MS(ES$^-$): 378/376 (M-1); ^{1}H-NMR(DMSO/DCL): 7.51 (s, 2H); 4.52, 4.38 (2d, 2H); 3.53 (m, H); 1.45 (dd, Me).
15. Optical purities determined by chromatography (e.g. **1f** and **2b**: e.e. > 99.8%, Chiralcel OD-R column).
16. The tetrazole derivative was obtained by alkylation of **6c** with 2-aminopropionitrile, followed by overnight treatment with $TMSN_3/Bu_2SnO$ in refluxing toluene, and deprotection with HBr in acetic acid (22% yield).