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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. © 1999 Elsevier Science Ltd. All rights reserved.

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<sup>1</sup>, amyotrophic lateral sclerosis<sup>2</sup>, anxiety<sup>3</sup>, epilepsy<sup>4</sup>, Parkinson's disease<sup>5</sup> and cerebral ischemia<sup>6</sup>.

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

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)<sup>9</sup>, in comparison to previously published analogues<sup>10</sup>.



a)  $IC_{50}$  in the [<sup>3</sup>H]AMPA binding assay<sup>13a</sup>; b)  $IC_{50}$  or % inhibition at 1µM in the [<sup>3</sup>H]-(Z)-2-carboxy-4.6-dichloroindole-3-(2<sup>-</sup>phenyl-2<sup>-</sup>carboxy)-ene ([<sup>3</sup>H]MDL-105519) binding assay<sup>13b</sup>; 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 ( $\pm$  3, Scheme 1) was obtained directly from ethylamine, then protected as its benzyl carbamate  $\pm$  4. The latter was resolved on a 500g-scale using simulated moving-bed chromatography<sup>11</sup>, and hydrogenated to the unstable optically active phosphoalanine 3<sup>12</sup>.

## Scheme 1



**Reagents and conditions:** a) i. MeCHO, ii. P(OMe)<sub>3</sub>, 77% after distillation; b) BnOOC-Cl, Et<sub>3</sub>N, 92%; c) resolution by simulated moving-bed chromatography on Chiralcel OJ, e.e. = 99%, yield = 35%; d) H<sub>2</sub>, 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<sub>3</sub>, 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<sub>3</sub>N, EtOOC-COCl, 3°C, 89%; b) H<sub>2</sub>SO<sub>4</sub>, KNO<sub>3</sub>, 0°C, 88%; c) TiCl<sub>3</sub>, aq. HCl, acetone, 0°C. 94%; d) i. POCl<sub>3</sub>, PCl<sub>5</sub>, reflux, 85%; ii. MeOH, MeONa, reflux, 75%; e) NBS, AIBN, C<sub>6</sub>H<sub>6</sub>, reflux, 2h, 67%; f) **3**, DMF, NaHCO<sub>3</sub>, 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'<sub>2</sub>O<sub>3</sub>P-alkyl-NH<sub>2</sub>, CH<sub>2</sub>Cl<sub>2</sub>, MgSO<sub>4</sub>, RT, 18h, then NaBH<sub>3</sub>CN, 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 5bromomethyl 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<sub>2</sub>-SH, THF, RT, 18h, ; b) 33% HBr in AcOH, 130°C; c) LiOH, THF, H<sub>2</sub>O, 46% from **6b**; d) H<sub>2</sub>O, dioxane, CaCO<sub>3</sub>, reflux, 24h; e) Bu<sub>4</sub>N<sup>+</sup>HSO<sub>4</sub>, CH<sub>2</sub>Cl<sub>2</sub>, 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 <sup>1</sup>H-NMR(250 MHz) and mass spectroscopy<sup>14</sup>

#### **Results and discussion** (Table 1)

Amino acid derivatives. Compound **8b**, formally derived from the selective AMPA antagonist **8a**<sup>10a</sup> 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<sup>15</sup> 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<sup>16</sup> had little effect on *in vitro* potency (IC<sub>50</sub> 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** ( $ED_{50} = 19 \text{ mg/kg}$ ) 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 ( $ED_{50} = 3 \text{ mg/kg}$ ), 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<sup>13</sup> 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  $\beta$ -phosphoalanine derivative 1h is less potent than its shorter analogue 1a, but is more selective. Introduction of a methyl group on its  $\beta$  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<sub>3</sub> 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<sup>7e and ref. cited therein</sup>.

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 <sup>a,f</sup>	MDL <sup>b,f</sup>	ESM <sup>c</sup>		Х	R	AMPA <sup>a,f</sup>	MDL <sup>b,f</sup>	ESM <sup>c</sup>
8a	NO <sub>2</sub>	HOOC	0.07	3.9	44	1a	NO <sub>2</sub>	H <sub>2</sub> O <sub>3</sub> PNH	0.29	1.0	7
8b	NO <sub>2</sub>	HOOC	0.61	9%	n.t.	1b <sup>d</sup>	NO <sub>2</sub>	H <sub>2</sub> O <sub>3</sub> P NEt	0.12	24%	18
8c <sup>d</sup>	NO <sub>2</sub>	HOOC	0.38	19%	35	1c	NO <sub>2</sub>	H <sub>2</sub> O <sub>3</sub> P NH	0.17	0.032	3
8d <sup>d</sup>	NO <sub>2</sub>	HOOC	0.16	0.76	32 (15 min)	1d	NO <sub>2</sub>	H <sub>2</sub> O <sub>3</sub> P NH	0.38	51%	8
8e <sup>d</sup>	NO <sub>2</sub>	HOOC	0.31	1.7	19, 0% (1h)	1e	NO <sub>2</sub>	H <sub>2</sub> O <sub>3</sub> P NEt	0.31	25%	26
8 <b>f</b> <sup>d</sup>	NO <sub>2</sub>		0.38	1.3	0%	1f	NO <sub>2</sub>	H <sub>2</sub> O <sub>3</sub> P NEt	0.08	13%	7. 18 (2h)
8g°	NO <sub>2</sub>	HOOC	0.29	-9% <sup>g</sup>	0%	1g	NO <sub>2</sub>	H <sub>2</sub> O <sub>3</sub> P NAc	1.3	31%	n.t.
8h <sup>d</sup>	NO <sub>2</sub>	HOOC	1.2	34%	0%	1h <sup>d</sup>	NO <sub>2</sub>	H <sub>2</sub> O <sub>3</sub> P	0.64	8%	18
8i <sup>d</sup>	NO <sub>2</sub>		0.34	22%	0%	1i°	NO <sub>2</sub>	H <sub>2</sub> O <sub>3</sub> P	0.20	6%	9
8j <sup>d</sup>	NO <sub>2</sub>	MeOOC	1.9	-3% <sup>8</sup>	n.t.	1j°	NO <sub>2</sub>	H <sub>2</sub> O <sub>3</sub> P	0.20	15%	10
9a <sup>d</sup>	Br	HOOC	4.7	0.04	0%	2a	Br	H <sub>2</sub> O <sub>3</sub> P NH	2.4	0.1	0%
9b	Br	HOOC	4.5	0.12	n.t.	2b	Br	H <sub>2</sub> O <sub>3</sub> P NH	3	0.006	12, 18 (2h)
9c <sup>d</sup>	Br	HOOC	4.3	2.2	n.t.	2c	Br	H <sub>2</sub> O <sub>3</sub> P NH	1.4	0.37	43
9d	Br	HOOC	3.8	37%	n.t.	2d	Br	H <sub>2</sub> O <sub>3</sub> P NH	16%	0.045	20%
14b	Br	$^{\rm HOOC}$ $\sim$ $^{\rm S}$	22%	0.12	0% (1h)	7 <b>a</b>	CF <sub>3</sub>	H <sub>2</sub> O <sub>3</sub> PNH	2.0	0.3	30
17	Br	ноос 🔨 о	4%	0.11	0% (1h)	7b°	CF <sub>3</sub>	H <sub>2</sub> O <sub>3</sub> P NH	1.2	0.006	8
18a	NO <sub>2</sub>	HOOCO	2.0	0.31	n.t.	7c°	CF <sub>3</sub>	H <sub>2</sub> O <sub>3</sub> P NH	0.54	0.59	20
18b	NO <sub>2</sub>	ноос үо	1.3	0.42	n.t.	7d	CF <sub>3</sub>	H <sub>2</sub> O <sub>3</sub> P NEt	0.068	4	10

a: [<sup>3</sup>H]-AMPA binding assay<sup>13a</sup>; b: [<sup>3</sup>H]-(Z)-2-carboxy-4,6-dichloroindole-3-(2'-phenyl-2'-carboxy)-ene ([<sup>3</sup>H]MDL-105519) binding assay<sup>13b</sup>; c) ED<sub>50</sub> [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<sub>50</sub> ± SEM in  $\mu$ M (from 6 or 12 concentrations of each compound), or % inhibition at 1  $\mu$ M; g) negative data within the normal variation range of results for inactive compounds. Acknowledgment: The authors thank C. Boesch, A. Koblet, M. Roggwiller, R. Haener and P. Schmid for their assistance, as well as E. Francotte and P. Richert for chromatographic support. Thanks also to P. Martin and N. Reymann for running the radioligand binding assays, and to C. Portet and A. Jeker for the anticonvulsion tests.

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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.



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- 14. E.g. **2b** (mw = 378.12): MS(ES<sup>\*</sup>): 378/376 (M-1); <sup>1</sup>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<sub>3</sub>/Bu<sub>2</sub>SnO in refluxing toluene, and deprotection with HBr in acetic acid (22% yield).