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5-Phosphonomethylquinoxalinediones as Competitive NMDA Receptor Antagonists with a Preference for the Human 1A/2A, Rather than 1A/2B Receptor Composition

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Abstract—NMDA antagonists derived from 5-phosphonomethyl-1,4-dihydroquinoxaline-2,3-dione (**3a**) are potent anticonvulsant agents, and display strong protective effects in the electroshock-induced convulsion assay in mice. Their preference for the human NMDAR 1A/2A over 1A/2B subunit composition was optimized, leading to (1*RS*,1′*S*)-PEAQX (**9r**), which shows a >100-fold selectivity.

In the course of our studies on substituted 5-methylquinoxalinediones, we identified **3a** as a potent, achiral, orally active NMDA antagonist with a 15-fold preference for human NMDA receptors with the 1A/2A, rather than 1A/2B, subunit composition. As no selective NMDA 1A/2A receptor antagonist has been described to date, we were interested in improving this preference, measured by electrophysiological recordings in *Xenopus* laevis oocytes expressing either 1A/2A or 1A/2B heteromeric human NMDA (hNMDA) receptors.[1] Three series of 5-phosphonomethylquinoxalinediones were synthesized for this purpose (Fig. 1), ultimately leading to **9r** ((1*RS*,1′*S*)-PEAQX), which shows a >100-fold preference for hNMDA 1A/2A receptors (Fig. 2).

In contrast to previously described competitive NMDA antagonists,[2] compounds of the first series show that chirality is not required for subnanomolar affinity at NMDA receptors. The addition of a chiral side chain, however, markedly improves the distinction betweeen hNMDA receptors composed of 1A/2A or 1A/2B subunits.

This article describes the binding affinity of these novel 5-phosphonomethylquinoxalinediones, as well as their functional selectivity in *Xenopus* oocytes expressing heteromeric hNMDA 1A/2A or 1A/2B receptors, and their anticonvulsant properties in the mouse electroshock-induced seizure test.

Chemistry

α-Unsubstituted 5-phosphonomethylquinoxalinediones were obtained from 5-bromomethylquinoxalines, for example **1**[3a] (Scheme 1), which reacted with trimethylphosphite to yield dimethyl phosphonate **2**, and after acidic hydrolysis 5-phosphonomethylquinoxalinedione **3d**. Alternatively, hydrogenolysis or replacement of the bromine atom in a Suzuki reaction yielded, after hydrolysis, **3a** or **3e**. The 7-chloro and 7-fluoro analogues (**3b** and **3c**) were prepared from the corresponding bromomethylquinoxalines.[3b,c]

α-Hydroxy-, methoxy- or acetoxy-5-phosphonomethylquinoxalinediones were obtained from 5-carbaldehydes (Scheme 2). For instance, **4**[3b] reacted with phosphinic acid dimethyl trimethylsilyl ester[4] to yield α-hydroxyphosphonate **5b**, which was deprotected to quinoxalinedione **6b** in concentrated HCl, or methylated before deprotection to yield **6c**. Acylation of **6b** with acetic anhydride yielded **6d**.

α-Amino-5-phosphonomethylquinoxalinediones were obtained by addition of phosphinic acid dimethyl trimethylsilyl ester to an imine, as exemplified in Scheme 3 for aldehyde **7** and methylamine, yielding **8b**. This

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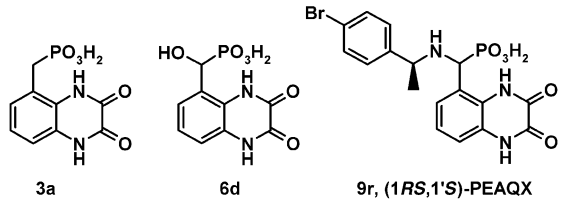


Figure 1. 5-Phosphonomethyl-1,4-dihydroquinoxaline-2,3-diones.

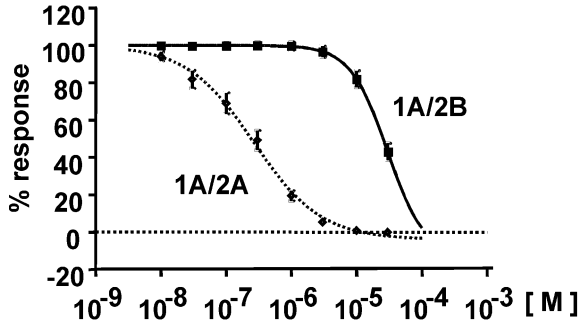
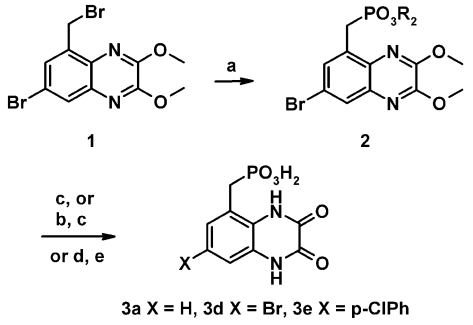


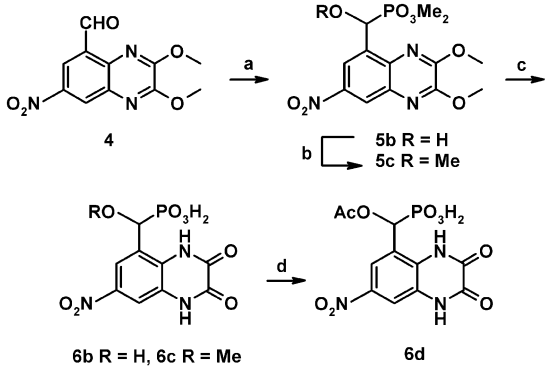
Figure 2. Effect of **9r** ((1*RS*,1′*S*)-PEAQX) on NMDA-induced currents in *Xenopus* oocytes expressing hNMDAR 1A/2A or 1A/2B. Data show mean value ± SEM ($N = 3$ or more).



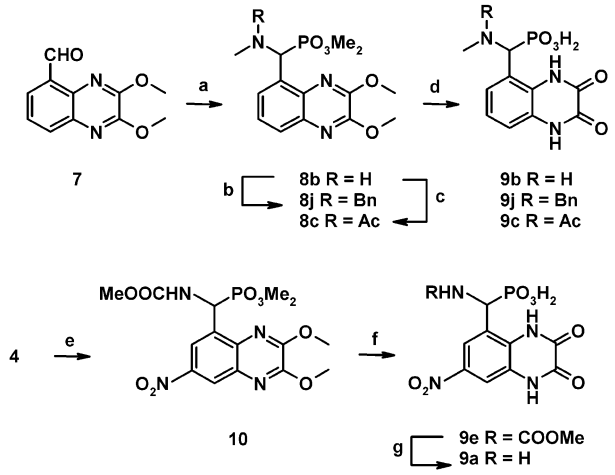
Scheme 1. 5-Phosphonomethylquinoxalinediones. Reagents and conditions: (a) $P(OR)_3$, reflux, 98% (**3a**: R = Me; **3e**: R = Et); (b) H_2, 10% Pd/C, Et_3N, MeOH; (c) AcOH, 12 N HCl, reflux, 20 h, 64%; (d) *p*-ClPhB$(OH)_2$, $(Ph_3P)_4Pd$, Na_2CO_3, $MeOCH_2CH_2OMe$, H_2O, 24 h, reflux, quant; (e) (i) TMSBr, CH_2Cl_2 then EtOH; (ii) HBr/AcOH, 66%.

intermediate can be benzylated (**8j**) or acylated (**8c**) before deprotection under acidic conditions. Primary α-amino phosphonates were prepared by condensation with methyl carbamate and dimethylphosphite,[5] which gave with aldehyde **4** the protected aminophosphonate **10** in good yield. A partial deprotection was obtained after a 20 h treatment with 4 N HCl at 100 °C, leaving the methylcarbamate mostly intact (**9e**). A prolonged treatment led to the fully deprotected α-aminophosphonate **9a**.

The methyl- and benzylphosphinates **11a**–**c** (Table 1) were prepared like the corresponding phosphonates, using benzyl- or methylphosphinic acid ethyl ester in place of dimethylphosphite. 5-Carboxymethyl-1,4-dihydroquinoxaline-2,3-dione (**12**) was synthesized from **1** in 15% yield by reaction with Bu_4NCN in CH_2Cl_2/H_2O at room temperature, hydrolyzed and deprotection under acidic conditions ($AcOH/H_2SO_4/H_2O$ 1:1:1), followed by catalytic debromination on 10% Pd/C in the presence of triethylamine.



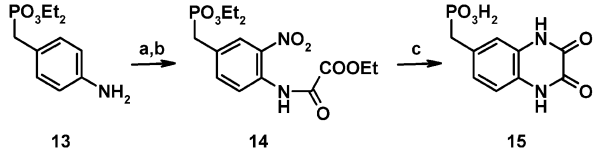
Scheme 2. α-Hydroxy-5-phosphonomethylquinoxalinediones. Reagents and conditions: (a) $(MeO)_2PHO$, TMSCl, Et_3N, CH_2Cl_2, 0 °C, 1 h, then **4**, rt, 4 h, 56%; (b) NaH, THF, then Me_2SO_4, 42%; (c) 2 N HCl, 100 °C, 18 h, 33–100%; (d) Ac_2O, 70 °C, 18 h, 46%.



Scheme 3. α-Aminoxy-5-phosphonomethylquinoxalinediones. Reagents and conditions: (a) (i) $MeNH_2{\cdot}HCl$, $MgSO_4$, K_2CO_3, CH_2Cl_2; (ii) $(MeO)_2PHO$, TMSCl, Et_3N, 0 °C to rt, 20 h; (b) BnBr, MeCN, iPr_2Net, 60%; (c) Ac_2O, quant; (d) 4 N HCl, reflux, 18 h, or 33% HBr in AcOH, 40 °C, 12 h, 75–95%; (e) $(MeO)_2PHO$, $MeOOCNH_2$, AcOH, $SOCl_2$, 86%; (f) 4 N HCl, 100 °C, 20 h, 44%; (g) 4 N HCl, 100 °C, 24 h, 68%.

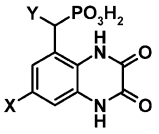
Finally, 6-phosphonomethylquinoxalinedione (**15**) was obtained by reaction of ethyl oxalyl chloride on diethyl *p*-aminobenzylphosphonate, followed by nitration and reductive cyclization (Scheme 4).

All new compounds showed satisfactory ^1H NMR (400 MHz) and MS analyses.

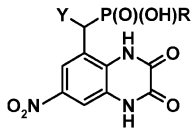


Scheme 4. 6-Phosphonomethylquinoxalinedione. Reagents and conditions: (a) EtOOC–COCl, pyridine, 37%; (b) NH_4NO_3, $(CF_3CO)_2O$, 88%; (c) $TiCl_3$, acetone, water.

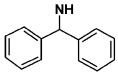
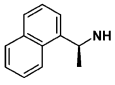
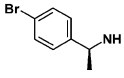
Table 1. In vitro affinities, functional selectivity in *Xenopus* oocytes and anticonvulsant effect in the MES test



3a-e Y = H; 6a-d Y = OR; 9a-s Y = R_1R_2N



11a R = Bn; 11b,c R = Me

Compd	X	Y	CGP 39653[a]	Oocytes: hNMDAR[b] 1A/2A	1A/2B	ratio	MES[c] ED_{50}
3a	H	H	0.5	2	30	1:15	0.7
3b	F	H	1.0	—	—	—	n.t.
3c	Cl	H	1.7	—	—	—	2.4
3d	Br	H	1.5	—	—	—	2.5
3e	*p*-ClPh	H	0.8	0.2	1.8	1:9	0.7
6a	H	HO	1.5	1.1	0.7	1:1	0.5
6b	NO_2	HO	1.2	—	—	—	1.2
6c	NO_2	MeO	17	—	—	—	n.t.
6d	NO_2	AcO	8.1	—	—	—	5
9a[e]	NO_2	H_2N	40	—	—	—	11.6
9b	H	MeNH	1100	790	6200	1:8	n.t.
9c	H	Me(Ac)N	860	2800	24,000	1:9	n.t.
9d[e]	NO_2	PrNH	65	90	930	1:10	2.8
9e	NO_2	MeOCONH	370	110	1400	1:13	8.8
9f[d]	NO_2		4.1	—	—	—	3.7
9g[d]	H	BnNH	2	4.2	34	1:8	2
9h[d]	NO_2	BnNH	1.5	—	—	—	1.5
9i[e]	NO_2		3	—	—	—	4
9j	H		160	600	30,000	1:50	40%
9k	H	PhNH	130	490	6200	1:8	0%
9l	H	$PhCH_2CH_2NH$	7.4	40	1600	1:40	10
9m	H		38	170	1500	1:9	2.3
9n	**H**		31	50	570	1:11	60%
9o	H		9.7	18	900	1:50	6.7
9p	H		120	60	820	1:14	2
9q	H		27	1700	> 30,000	> 1:17	15
9r	H		8	270	29,600	1:111	23
11a[d]	NO_2	BnNH	60	50	1300	1:26	20%
11b[d]	NO_2	BnNH	80	200	1300	1:7	0%
11c	NO_2	HO	42%	—	—	—	0%

[a]IC_{50} (nM) or % inhibition at 1 μM in the [^{3}H]CGP39653 binding assay,[6] average of at least two experiments run in triplicate.
[b]IC_{50} (nM) based on at least six concentrations of test compound; $n = 3$ or more.
[c]Mouse electroshock-induced seizure test, ED_{50} (mg/kg) or % protection at 50 mg/kg, 1 h after ip administration; $n = 5$.
[d]HBr salt.
[e]HCl salt.

Biological Activity

The parent structure, 5-phosphonomethylquinoxalinedione (**3a**), is a highly potent antagonist with subnanomolar affinity in the [^{3}H]CGP39653 binding assay[6a] for NMDA receptors. In vivo, it shows a very long duration of action, with an ED_{50} value in the mouse electroshock-induced seizure test (MES)[7] of 1.1 mg/kg, 8 h after ip administration, and 6.7 mg/kg, 8 h after oral administration. Variation of the aromatic substitution on C(7) (**3b–e**) shows that this position is largely insensitive to steric as well as to electronic effects, a pattern not seen with quinoxalinediones acting at AMPA receptors[3a,e] or at the glycine-binding site of NMDA receptors.[3d,e] Replacement of the phosphonic acid by a carboxylate (**12**) strongly decreased in vitro affinity (IC_{50} = 250 nM), and abolished activity in the electroshock-induced convulsion model in mice up to 50 mg/kg (ip, 1 h). Of note, 6-phosphonomethyl-quinoxalinedione (**15**), was completely inactive at hNMDA receptors. Compounds in Table 1 have no binding affinity for AMPA receptors,[8] and at least a 250-fold selectivity with regards to the glycine-binding site of NMDA receptors[9] (data not shown).

Introduction of a hydroxy group in the benzylic position (**6a**,**b**) maintains both affinity for hNMDA receptors and in vivo potency, with *O*-acylation (**6d**) or *O*-methylation (**6c**) having only minor effects on affinity. Compound **6a** does not differentiate between hNMDA 1A/2A and 1A/2B receptors subunit compositions.

In the α-aminophosphonic acid series, *N*-substitution by a relatively bulky group like benzyl (**9g**) or cyclohexylmethyl (**9i**) appears necessary to reach low nanomolar binding affinity. An additional methyl group on the nitrogen strongly decreased in vitro and in vivo potency (**9j**), while allowing a 50-fold discrimination between hNMDA 1A/2A and 1A/2B subunit combinations.

In terms of receptor affinity, the position of the phenyl ring seems optimal in the benzylamine derivative **9g**, which is more potent than the aniline (**9k**) and phenethyl analogues (**9l**). Introduction of an additional alkyl group in the benzylic position decreased affinity 5- to 15-fold (**9m–o**).

In terms of subunit selectivity however, introduction of a methyl group in this position has a significant effect. The (*R*)-phenethyl derivative (**9n**) shows no increase in selectivity as compared to **9g**, but the (*S*)-phenethyl stereoisomer (**9o**) displays a 50-fold preference for hNMDA receptors composed of the 1A/2A subunit combination. Finally, introduction of a bromine atom in the *para* position of the phenethyl group led to **9r** [(1*RS*,1′*S*)-PEAQX], which has a high binding affinity for NMDA receptors (IC_{50} = 8 nM), and a functional preference in excess of 100-fold for hNMDA 1A/2A (IC_{50} = of 270 nM) over 1A/2B receptors (IC_{50} = 29,600, Fig. 2). The separation of the (*RS*)- and (*RR*)-diastereomers and their pharmacological properties will be described in detail elsewhere.

The in vivo potency in the MES test appears to be independent of the affinity for hNMDA receptors composed of 1A/2B subunits. For instance, **3a** and **6a**, which have similar affinities in the radioligand binding assay, display identical potencies in the MES test, despite very different IC_{50} values at hNMDA 1A/2B receptors. The most selective compound, (1*RS*,1′*S*)-PEAQX (**9r**), which is practically inactive in *Xenopus* oocytes expressing hNMDA 1A/2B receptors, displays an ED_{50} value of 23 mg/kg in the MES test.

In contrast to their phosphonate analogues, phosphinates (**11a–c**) have weaker binding affinity to hNMDA receptors. **11a** shows a 26-fold preference for the 1A/2A subunit composition, and is like **11b** and **11c** inactive in the MES test.

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