

5-AMINOMETHYLQUINOXALINE-2,3-DIONES.

PART I: A NOVEL CLASS OF AMPA RECEPTOR ANTAGONISTS.

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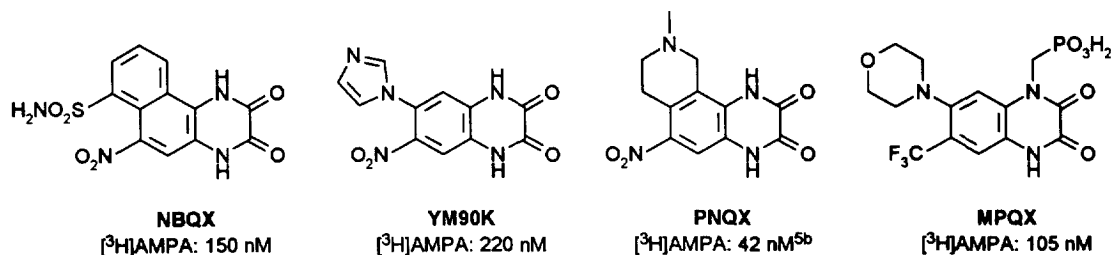
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Abstract: A series of 5-aminomethylquinoxaline-2,3-diones have been identified as potent and selective AMPA antagonists. Some of these compounds are also active at the glycine-binding site of the NMDA receptors. A number of these novel, water-soluble quinoxaline-2,3-dione derivatives display protective effects in the electroshock-induced convulsion model in mice. © 1997 Elsevier Science Ltd. All rights reserved.

The neurodegeneration and cell death following cerebral ischemia have been linked to an excessive glutamate release, which initiates excitotoxic damage via overactivation of several ionotropic receptors¹. This process is mediated by NMDA, AMPA and kainate-preferring glutamate receptors, and blocking their activation is expected to have a neuroprotective effect. AMPA antagonists are of particular interest, as they have been shown to be neuroprotective in animal models of focal² and global³ cerebral ischemia, without the side-effects associated with NMDA receptors blockade. They can also prevent the excessive neuronal activation by glutamate receptors responsible for epileptic seizures⁴.

The quinoxaline-2,3-diones represent a well-known class of AMPA receptors antagonists⁵. Except for the recently disclosed MPQX^{5c}, other published compounds (Fig. 1) suffer from a limited *in vivo* activity, due to a low bioavailability or a short duration of action.

Figure 1: Most prominent AMPA antagonists of the quinoxaline-2,3-dione type.

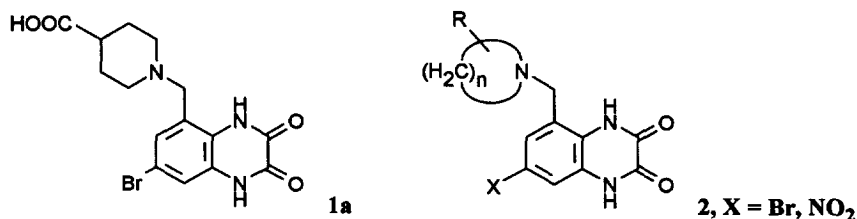


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Screening of the in-house chemical collection allowed the identification of **1a**, a moderate, albeit selective AMPA antagonist with a good water solubility. As it has previously been shown that 7-nitro-, rather than 7-bromo-quinoxaline-2,3-diones are potent AMPA antagonists^{5b,c}, several analogs of **1a** with this substitution were synthesized. The structure-activity relationship of the resulting 5-aminomethylquinoxaline-2,3-diones bearing a cyclic amine (**2**) will be described in this article.

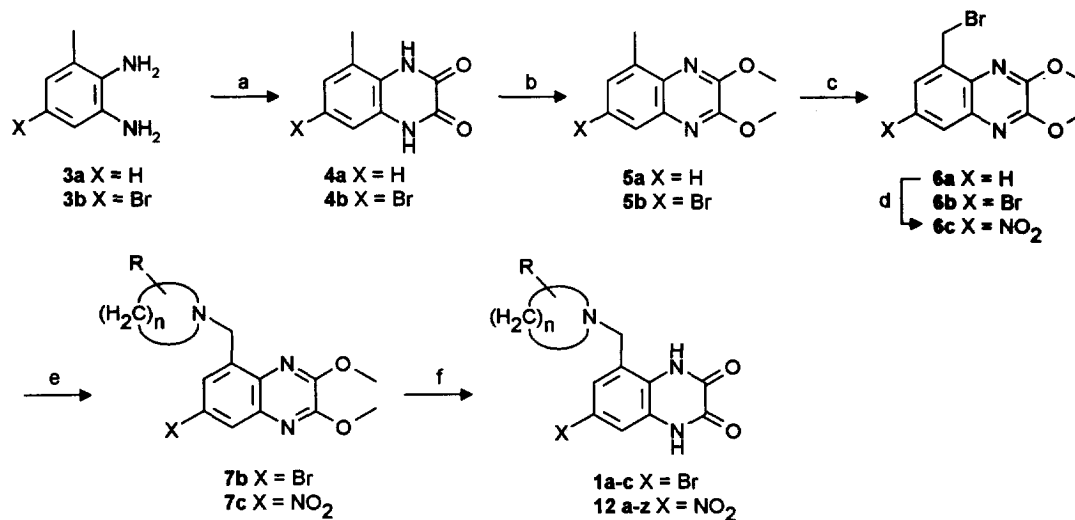
Figure 2



Chemistry

5-Methylquinoxaline-2,3-dione **4a**⁶ was prepared from **3a** and oxalic acid, then protected as its dimethoxy derivative **5a** by treatment with POCl₃ followed by methanolysis. Benzylic bromination with NBS and nitration gave a mixture of 7- and 8-nitro isomers, from which **6c** was crystallized with a 38% yield. The 7-bromo derivative **6b** was obtained from **3a** via a similar procedure. Alkylation of cyclic secondary amines with **6b** or **6c**, followed by acid hydrolysis, gave the desired compounds with good yields (scheme 1).

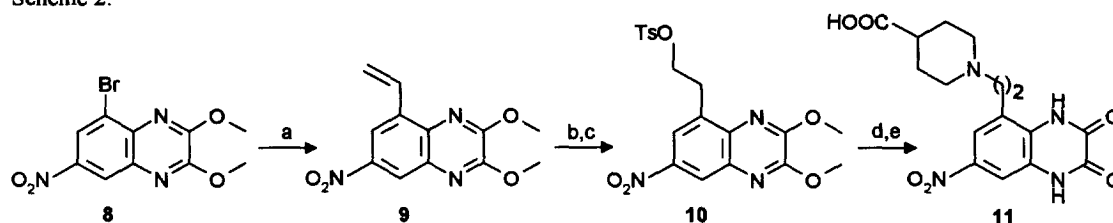
Scheme 1



Reagents and conditions: a) (COOH)₂, 2N HCl, reflux, 18h, 98% b) i: POCl₃, reflux, 18h. ii: MeOH, MeONa, reflux, 18h, 94%; c) NBS, AIBN, CCl₄, reflux, 24h, 80%; d) H₂SO₄, CF₃COOH, KNO₃, 38%; e) cyclic amine, (i-Pr)₂NEt, MeCN, reflux, 82-98%, except 2-methyl-aziridine or azetidine, CH₂Cl₂, aq. 40% Bu₄NOH, 92% or 99%; f) 24% HBr/AcOH, 50°C, 4h or 2N HCl, reflux, 4-18h, 52-99%.

Tosylate **10** was obtained via the palladium-catalyzed coupling of **8** with vinylbutylstannane, followed by hydroboration with 9-BBN, oxidative work-up and tosylation. **10** reacted with ethyl piperidine-4-carboxylate to yield **11** after deprotection with refluxing 2N HCl.

Scheme 2:



a) $\text{Bu}_3\text{SnCHCH}_2$, $\text{Pd}(\text{PPh}_3)_4$, toluene, 110°C , 75%; b) 9-BBN, then H_2O_2 , aq. Na_2CO_3 , 59%; c) TsCl , pyridine, 30%; d) ethyl piperidine-4-carboxylate, 110°C , no solvent; e) 2N HCl, reflux, 8h, 14% from **10**.

Results and discussion

We first studied the influence of the carboxylic acid group in our lead compound (**1a**) through its displacement on the piperidine ring. The resulting structures (**1b,c**) have weaker affinities for AMPA receptors. The 7-bromo substituent was subsequently replaced by a nitro group to yield **12a**, which displayed the most potent *in vitro* activity within this series. Converting this compound to an ester (**12b**) or an amide (**12c**) diminished its affinity. Interestingly, the corresponding 7-bromo-8-nitro derivative **13**⁷ is inactive.

Removal of the carboxylic acid group of **12a** affected its binding potency only to a limited extent (**12d**). Other substituents in this position did not improve the affinity for AMPA receptors (**12e-i**), although replacement of the carboxylate by a phenyl group (**12e**) showed that there are minimal steric constraints in this position. Piperidine derivatives with small groups on positions 4 (**12g-i**) or 3 (**12j-l**) maintained an almost constant affinity, regardless of the nature of the substituent.

Through systematic variations of the ring size from 3 to 7 members (**12d, m-p**), it was shown that the best binding affinities could be obtained with the pyrrolidine and piperidine derivatives. The introduction of a double bond in these cycles had little influence (**12q-r**), whereas opening the piperidine ring decreased the potency slightly (**12s**). The insertion of a second heteroatom within the saturated ring decreased the affinity (**12t-v**), especially for piperazine. Pyrrolidine derivatives did not display better affinities for AMPA receptors (**12w-z**). Of interest is that the 3-hydroxy-pyrrolidine derivative **12z** binds strongly to the glycine-binding site of NMDA receptors, in contrast to the parent pyrrolidine **12o**.

Homologation of the linker between the piperidine ring and the quinoxaline-2,3-dione nucleus of **12a** decreased the affinity for AMPA receptors to $1.6\ \mu\text{M}$ (compound **11**).

Table 1: Structures and *in vitro*⁸ affinities of the cyclic 5-aminomethylquinoxaline-2,3-diones

		AMPA ^a	NMDA (glycine) ^{a,b}
1a-c			
12a-z			
13			
		AMPA ^a	NMDA (glycine) ^{a,b}
1a		0.89 ± 0.12	0%
1b		2.5	24%
1c		18%	0.84 ± 0.11
12a^c		0.07 ± 0.02	3.9
12b^c		0.35 ± 0.04	0.59 ± 0.01
12c^c		0.76 ± 0.5	4%
12d^c		0.29 ± 0.09	41%
12e^c		0.43 ± 0.1	0.33 ± 0.06
12f^d		2.0	34%
12g^c		0.37 ± 0.13	31%
12h^d		0.31 ± 0.09	18%
12i^d		0.43 ± 0.29	45%
12j^d		0.52 ± 0.15	14%
12k^c		0.60 ± 0.19	3
12l^c		0.43 ± 0.14	4%
12m^c		2.2	40%
12n^c		1.5	3.8
12o^c		0.28 ± 0.13	0%
12p^c		0.58 ± 0.15	0%
12q^c		0.49 ± 0.13	36%
12r^c		0.36 ± 0.12	40%
12s^c		0.84 ± 0.25	0%
12t^c		0.66 ± 0.09	32%
12u		11	21%
12v		3.4	8%
12w^d		0.70 ± 0.11	24%
12x^c		0.46 ± 0.16	2
12y^d		2.1	1.5
12z^c		0.33 ± 0.02	0.28 ± 0.19
13^c	-	15%	14%

a: IC₅₀ ± SEM in μM or % inhibition at 1 μM; average of at least two triplicate experiments; b: [³H]-(Z)-2-carboxy-4,6-dichloroindole-3-(2'-phenyl-2'-carboxy)-ene ([³H]MDL-105519) or [³H]-DCKA binding assay c: HBr salt; d: HCl salt

The compounds with the best binding affinities were tested in the electroshock- and metrazole-induced convulsion assays in mice⁹ (Table 2). Activities remained modest, with ataxia being the most frequently observed side-effect at doses close to the ED₅₀. Interestingly, **12g** also proved active after oral administration (1h pretreatment, ED₅₀ = 54 mg/kg).

Table 2: compounds active in the anticonvulsion tests with an ED₅₀ below 50 mg/kg:

	ED ₅₀ (mg/kg) or % protection at 50 mg/kg after 30' (i.p. administration)	
	electroshock	metrazole
12a ^a	44	n.t.
12g ^a	33	16
12o ^a	40%	26
12q ^a	60%	35
12z ^a	34	n.t.

a: HBr salt; n.t.: not tested

In summary, the optimization of our lead compound **1a** led to the identification of the nitro derivative **12a**, a potent, selective AMPA antagonist with a good water solubility (1.68 g/L at pH = 7.4). Broad variations of the piperidine ring could not improve the binding affinity any further. Although potencies were moderate, several of these compounds were shown to be active in the electroshock- or metrazole-induced convulsion models in mice. Further 5-aminomethylquinoxaline-2,3-diones containing an acidic function have been synthesized, and will be the subject of a separate publication.

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