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Pyrrolylquinoxalinediones : A new class of AMPA receptor antagonists

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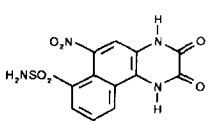
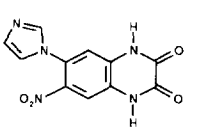
Abstract: Pyrrolylquinoxalinediones were synthesized and their affinities for the AMPA receptor were determined. Most compounds showed moderate to good affinities. The acetic acid derivative **8b** exhibited a K_i value of 70 nM and was equipotent to NBQX **1**. Structure activity relationships are discussed. Selected compounds were tested for their potency to inhibit AMPA induced lethal convulsions in mice. In this *in vivo* model the compounds showed improved potency compared with NBQX.

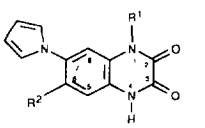
Glutamate represents the major excitatory neurotransmitter in the brain. A role of glutamate has been proposed for a number of pathophysiological conditions such as cerebral ischemia and epilepsy where the ionotropic glutamate receptors, particularly NMDA and AMPA receptors, are believed to be excessively activated. This may lead to fatal neuronal damage. Consequently antagonists for NMDA and AMPA receptors were considered candidates for clinical therapy[1,2]. The discovery of the quinoxalinediones such as DNQX and CNQX (for review see 3) was an important milestone in the development of selective high affinity AMPA receptor antagonists. On the basis of CNQX as lead structure other competitive AMPA antagonists with higher affinity and selectivity were synthesized. One of the first of these, NBQX, was used to demonstrate the effectiveness of AMPA antagonists in experimental stroke and epilepsy models[4]. Disappointingly its use was associated with drawbacks such as poor penetration of the blood brain barrier and poor water solubility which may cause serious side effects[5]. In order to circumvent the disadvantages of NBQX a number of synthesis programs were started using quinoxaline as lead structure[6]. Well known key structures derived from these programs are YM 90K[6d], NS 257 and LY 293558 all of them representing quinoxaline-like competitive AMPA antagonists.



NBQX (1)



YM 90K



Pyrrolylquinoxalinediones

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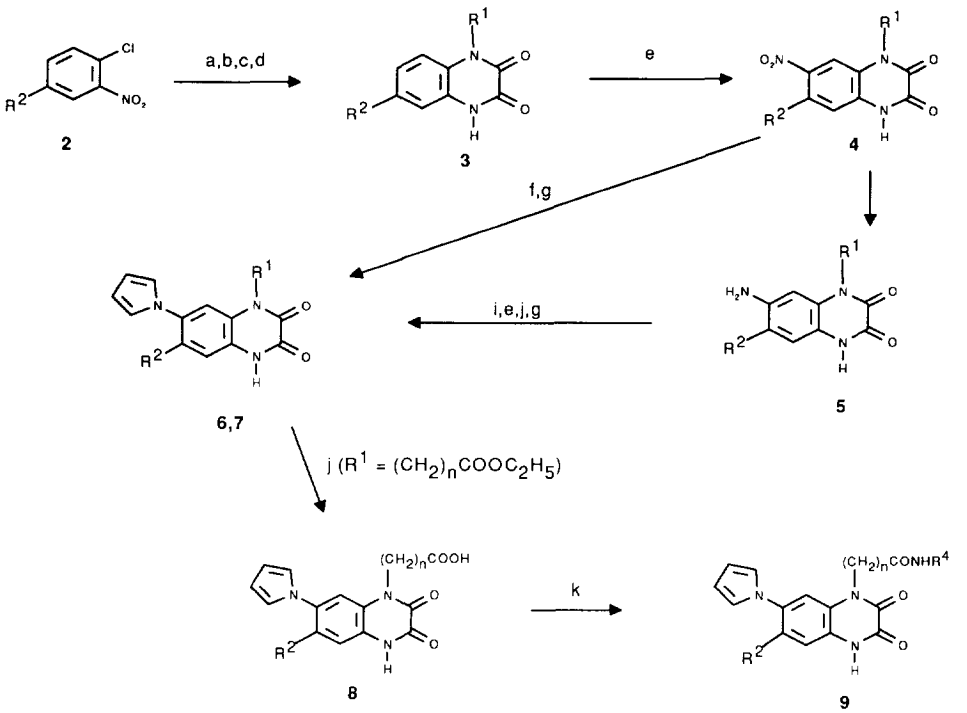


Scheme: a) R^1NH_2; - b) for $R^1 = (CH_2)_nCOOH$: EtOH, H_2SO_4; c) EtOOCCOCl; d) Fe, AcOH, reflux; e) KNO_3/H_2SO_4; f) H_2/ Pd(C); g) 2,5-dimethoxytetrahydrofurane, AcOH/reflux; h) $HCOONH_4$, Pd(C); - i) AcCl; j) HCl; k) R^4NH_2, DCC.

With the intention to improve the water solubility and brain availability of NBQX we also launched a synthesis program for new competitive NBQX-related AMPA antagonists. During this program we discovered the pyrrolylquinoxalinediones which are closely related to YM90K.

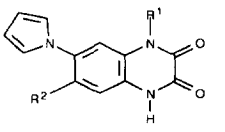
Quinoxalinediones (= QX) such as CNQX are known to be very polar heterocyclic compounds which poorly penetrate through blood brain barrier[7]. Generally, enhancing the lipophilicity may be an obvious strategy to improve blood brain penetration of polar compounds. Therefore we had chosen a pyrrol ring which may mimic a cyano or nitro group and contribute to lipophilicity as substituent at the quinoxalinedione ring. As expected, the replacement of the nitro group in 7-nitro-6-trifluoroquinoxaline-2,3-dione (log P_w < -2) by a pyrrolring (**6a**) increased the log P_w value by more than 1 (log P_w = -0.9)[8]. With the introduction of a cyclohexyl ring (**7a**) the log P_w value increases further to + 2.5. This finding encouraged us to synthesize and evaluate pyrrolylquinoxalinediones as potential AMPA antagonists with improved properties.

The chemical synthesis of the pyrrolylquinoxalinediones is outlined in Scheme 1. Aniline derivatives were prepared by aromatic nucleophilic substitution (reaction a) of the halogenated compounds **2** with R^1NH_2 and were transformed to the oxalic amides (reaction c). Reduction of the ortho-nitro group and ring closure to the quinoxalinediones **3** was performed in a one-pot procedure using iron in hot acetic acid (reaction d). The quinoxalinedions **3** were converted to the 7-amino derivatives by nitration followed by hydrogenation. A subsequent Paal-Knorr synthesis led to the pyrrolylquinoxalinediones **6** and **7**. The 6-nitro derivatives **6b**(R^2= NO_2) were synthesized by blocking the 6-position initial steps with a chloride **2**(R^2 = Cl) which was removed during reduction of **4** to **5** using ammonium formate under Palladium catalysis.

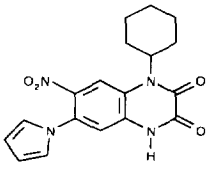
The 8-pyrrolylquinoxalinedione **11** was prepared by catalytic hydrogenation of the oxalic estermonoamide of the commercial available 2,6-dinitro-4-trifluoromethylaniline and subsequent pyrrolring formation. The cyclohexyl derivative **10**, with the arylsubstituents reversed was prepared from 1-chloro-2,4-dinitrobenzene followed by an analogous quinoxalinedione formation and pyrrolring introduction as above.

Table 1 : a) Receptor binding with specific radio labelled [^{3}H]-AMPA[9]. The K_i values are mean values for two or more independent experiments.

	R^1	R^2	Receptor binding [^{3}H]-AMPA K_i/µM [a]
6a	H	CF_2	3
b	H	NO_2	0.4
c	H	Cl	20
7a	c-C_6H_{11}	CF_3	1.6
b	CH_2CH_3	NO_2	0.85
c	c-C_6H_{11}	Cl	10
d	c-C_6H_{11}	NO_2	0.4
e	$CH_2CH(CH_2CH_3)_2$	NO_2	6
f	c-C_6H_{11}	SO_2CH_3	15
g	c-C_3H_5	NO_2	3.5
h	CH_2COOCH_3	CF_3	> 25
i	$CH_2COOCH_2CH_3$	NO_2	0.91
j	$CH_2CH_2COOCH_2CH_3$	CF_3	0.58
k	$CH_2CH_2COOCH_2CH_3$	NO_2	0.26
8a	CH_2COOH	CF_3	0.17
b	CH_2COOH	NO_2	0.07
c	CH_2CH_2COOH	CF_3	0.39
d	CH_2CH_2COOH	NO_2	0.33
9a	$CH_2CONHCH_3$	CF_3	20
b	$CH_2CH_2CONHCH_2Ph$	CF_3	8.0
c	$CH_2CONHCH_2CH_3$	NO_2	2.7
d	$CH_2CONHPh$	NO_2	2.1
e	$CH_2CONHCH_2Ph$	NO_2	20
f	$CH_2CONHCH_2CH_2Ph$	NO_2	20
10			25
11			> 25
NBQX			0.07



6 (R^1=H); **7**; **8**; **9**



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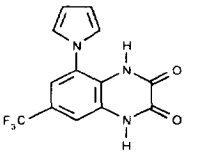

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The AMPA receptor affinity was determined by a binding assay described by Honoré *et al.* with slight modifications[9]. The results are shown in Table 1.

The position of the pyrrole substituent at the QX is important for receptor binding. 7-Pyrrolyl derivatives show moderate binding affinity (examples **6a**, **b** and **c**) whereas the 5-pyrrolyl derivative **11** fails to bind (i.e. K_i > 30000 nM). Furthermore, substituents of position 6 at the benzene ring showed modified affinity, which increased in the order Cl < CF_3 < NO_2[10]. The nitro derivative **6b** is 50 fold more potent in binding than the chloride derivative **6c**.

	AMPA[a] K_i/µM	Glycine[a] K_i/µM	Kainate[a] K_i/µM	*in-vivo* AMPA antagonism[b] ED_{50} (mg/kg) 15 min.[c]	60 min.[c]
6b	0.4	10	4.9	21	31
7b	0.85	nt.[d]	nt.	> 50	12
7d	0.4	nt.	nt.	> 50	22
7i	0.91	nt.	nt.	> 50	26
7k	0.26	>30	8.5	> 30	14
8a	0.17	8.0	1.5	> 50	> 50
8b	0.07	> 25	0.41	30	30
8c	0.39	1.2	nt.	> 50	50
8d	0.33	> 30	5.2	> 30	30
NBQX	0.07	33	2.6	50	> 50

Table 2 : a) Receptor binding with specific radio labelled ligands ^{3}(H)-AMPA [9], ^{3}(H)-glycine [10] or [^{3}H]-Kainate. The affinity constants K_i are mean values of two or more independent experiments; b) ip. doses of the compound which protect 50% of the animals ; c) Time at which the compounds were administered ip. prior to application of AMPA icv. d) not tested.

A substitution at the nitrogen atoms of pyrrolylquinoxalinediones (either position 1 or 4) two isomers could give two isomers which may differ in receptor affinity. For example, the cyclohexyl derivative **7d** was 70 fold more potent in displacing [^{3}H]-AMPA than **10**. Further examples (data not shown) support the observation that 1-substituted 7-pyrrolylquinoxalindiones were the most favorably structural substitutions to improve receptor binding[11].

Certainly, the alkyl or cycloalkyl residues we used as substituents at the quinoxalinedione nitrogen enhance the lipophilicity but they do not improve receptor affinity. On the contrary, apart from the cyclohexyl residue all alkyl groups used as substituents decrease binding. These alkyl residues differentiate between the AMPA receptor and the glycine binding site at the NMDA receptor. Introduction of any alkyl residue at the 1-position diminishes glycine binding affinity (see table 2). This is illustrated by comparing **6a** (K_i=8.5µM) with **7a** (>25µM) and **6b** (10µM) with **7d** (>25µM).

It has been reported that acid residues at the 1-nitrogen may be favorable for receptor binding and this corresponds to our results [2]. The acetic esters **7h, 7i** exhibit poor or moderate affinities whereas the acetic acid residues in **8a, 8b** represent high affinity ligands. The binding of the CF_3-derivative increases 16-fold (**6a**, Ki= 3µM versus **8a**, K_i = 0.18µM) and for the nitro derivatives still 5-fold (**6b** versus **8b**). The nitro acetic acid derivative **8b** has a K_i = 70nM which shows the highest affinity here reported and is equipotent to NBQX **1**. Homologation of the alkyl chain from a methylene group to the propionic acid derivatives has conflicting results. The acids **8c** and **8d** again are also potent ligands but there was no improvement over the acetic acid derivatives. In contrast, the propionic esters are as potent as the acids. Remarkably, the nitro ester **7k** is one of the most potent AMPA ligand presented here (K_i = 0.26µM). All amides **9** exhibit only poor or moderate affinity and there is no recognizable improvement of binding by the chain size and amide substituents .

To assess the AMPA antagonistic properties the compounds were tested for their ability to antagonize AMPA induced lethal convulsions in mice (see table 2). The compounds were administered intraperitonialy (ip.) either 15 or 60 min. before 40 nmol AMPA (dissolved in 10µL water) was injected intracerebroventricularly and ED_{50} values were calculated as the dose which protect 50% of the animals. The ED_{50} values are expected to reflect both the intrinsic activity as well as the ability of compounds to penetrate through blood brain barrier.
Our results confirm previous reports that NBQX exhibits only a short period of activity *in-vivo*[6]. If applied 5 minutes prior to AMPA the ED_{50} value is 16 mg/kg (data not shown). However a high dosage of 50 mg/kg is necessary for sufficient protection when the pretreatment period is extended to 15 minutes. Several pyrrolochinoxalindiones are more potent than NBQX in this model and are still effective when applied 60 minutes prior to AMPA. For a 15 minutes pretreatment period, only two compounds, **6b** and **8b** have ED_{50}'s below 50 mg/kg (= ED_{50} of NBQX). Both compounds are also effective after 60 minutes, indicating a longer time of *in-vivo* efficacy compared with NBQX. The more lipophilic compounds **7b**, **7d**, **7i** and **7k** are even more potent but obviously a prolonged time of pretreatment was required to be fully effective. The ethyl derivative **7b** and the propionic ester **7k** have ED_{50}s of 12 and 14 mg/kg ip., respectively, and are the most potent antagonists in the *in-vivo* AMPA antagonism shown here. The cyclohexyl derivative **7d** has an ED_{50} of 22 mg/kg demonstrating that a further increase of lipophilicity does not improve *in-vivo* efficacy. The acids **8a-8d** are less effective and show ED_{50} values of 30 and 50 mg/kg. The results suggest that compounds carrying polar groups are less active *in-vivo* than the lipophilic alkyl derivatives.

In summary, we discovered the pyrrolylquinoxalinediones as new competitive AMPA antagonists. Derivatives, particulary **8b**, exhibit good receptor affinity, comparable with that of NBQX **1**. Furthermore several pyrrolylquinoxalinediones were tested *in vivo* to evaluate their ability to penetrate the blood brain barrier. The results indicate improved *in vivo* efficacy and a markedly prolonged time of action.

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