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Pyrrolylquinoxalinediones : Dicarboxylates as highly potent AMPA receptor antagonists

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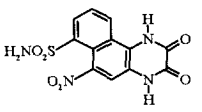
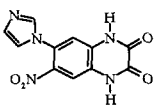
Abstract: Pyrrolylquinoxalinediones carrying carboxylates at the pyrrole side-chain were synthesized and evaluated for AMPA receptor binding and for selectivity over other glutamate receptors. Particularly dicarboxy derivatives represent selective and highly potent AMPA antagonists. Moreover, several compounds displayed a remarkable efficacy against AMPA induced lethal convulsions and maximal electroshock seizures (MES) in mice. The good *in vivo* efficacy of the highly polar compounds suggests the involvement of an active transport mechanism. © 1997 Elsevier Science Ltd.

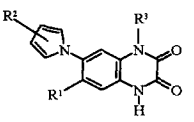
The glutamate receptors, including the AMPA receptor, have been suggested to be involved in neuronal cell damages caused by a cerebral ischemia as well as in epilepic seizures[1)]. Therefore antagonists of the AMPA receptor have been proposed for therapy of stroke and epilepsy[2)]. Indeed, it was demonstrated that the selective AMPA antagonist NBQX displayed efficacy in experimental stroke[3)] and in epilepsy models [4)]. During the last years a number of structurally distinct competitive AMPA antagonists were reported such as YM90K [5)], NS 257 [6)], LU 293558 [7)], PNQX [8)], S17625 [9)] as well as the first noncompetitive antagonist GYKI 52466 [10)].
Recently, we presented the pyrrolylquinoxaline-2,3-diones as a new class of competitive AMPA antagonists[11,12)]. We continued our efforts to provide selective, high affinity AMPA receptor antagonists. Furthermore, the desired compounds should show improved water solubility to allow an intravenous administration which is important for agents to be used in clinical stroke therapy. In this paper we present new dicarboxylates derived from pyrrolylquinoxalinediones that encompass these requirements and , surprisingly, disclose extraordinary *in vivo* efficacy.



NBQX **1**



YM90K **2**



Pyrrolylquinoxalinediones

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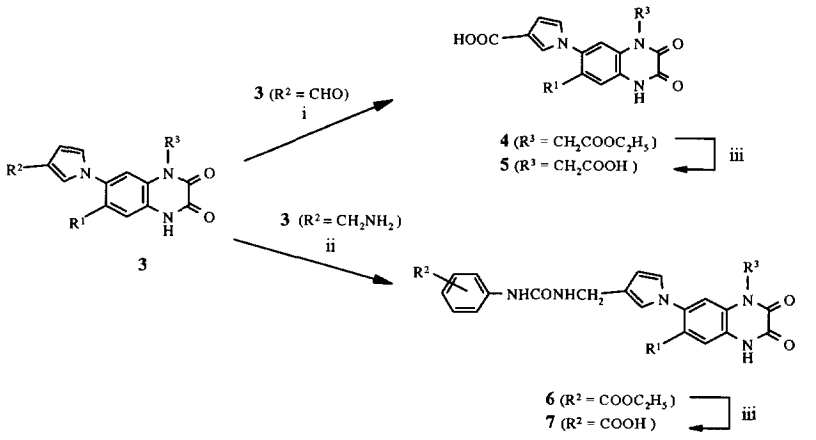


Scheme 1: methods: i) $KMnO_4$, dicyclohexano-8-crown-6, acetone, reflux (40-60%); ii) R^2-Ph-NCO, DMF; 50-90°C, 5-30minutes (60-90%); iii) LiOH, 25°C (80-90%);.

The synthesis of the pyrrolylquinoxalinediones is outlined in Scheme 1 and corresponds to the previously reported routes[12]. Pyrrole-3-aldehydes **3** (R^2 = CHO)[12] were oxidized by $KMnO_4$ in boiling acetone in the presence of dicyclohexano-18-crown-6 to afford the carboxylates **4** in moderate yields (30-70%)[13]. The ureas **6** were synthesized from **3** ($R^2 = CH_2NH_2$)[12] and appropriate phenylisocyanates in dimethylformamide at 80-100°C. In the final steps the ester residues were hydrolysed by LiOH in tetrahydrofuran/water mixtures at ambient temperature to provide the carboxylates **5** and **7** in high yields.

The AMPA receptor affinities of the compounds were determined in a [^{3}H]-AMPA displacement assay described by T. Honoré *et al.*[14]. Results are shown in Table 1.

The introduction of a carboxylate at the 3-position of the pyrrole ring enhances the AMPA receptor affinity. For example, the pyrrolylquinoxalinedione **4a** (K_i = 0.06μM) is 6-fold more potent than the parent compound **3a** (K_i = 0.4 μM). Likewise, the compounds **5a** and **5b** that carry an additional methylcarboxylate attached to the quinoxaline ring display 3-fold improved potency over the related derivatives **3c** and **3d**, respectively. As previously reported, compounds with alkylesters attached to quinoxaline ring display only moderate affinity (see **4b** and **4c**) [11].

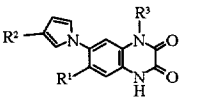
Except **6b** all ureas **6** and **7** are potent AMPA antagonists superior to NBQX and YM90K. Compared to the parent urea **6a** the substitution of the distal phenyl ring with ester or a carboxylate residues has only slight or no positive effect on binding. Furthermore, there is only a minor influence of the aromatic substitution pattern (compare **6c** and **6d**, **7b** and **7c**). Both monoesters **6c** and **6d** disclose the highest affinities (K_i's = 0.004μM) and both belong to the most potent AMPA antagonists discovered up to now. The importance of the methylcarboxylate residue attached to the quinoxaline nucleus is demonstrated by **6d** and **7c** which are >100fold and 7fold more potent than the corresponding dicarboxylates **6b** and **7a**, respectively.

The selectivity of the compounds for the AMPA receptor versus the glycine binding site at the NMDA receptor and versus the high affinity kainate binding site was evaluated by [^{3}H]-glycine[15] and [^{3}H]-kainate[16] displacement assays (see Table 2). All pyrrol derivatives exhibit high selectivity over both, NMDA and kainate receptors. For example, the urea derivative **7c** displays > 4000-fold selectivity over the glycine binding and 510-fold selectivity over the kainate binding. With respect to the pyrrolylquinoxalinediones reported recently[12], there is reasonable evidence that the carboxylate groups in the pyrrolic side chain may be a key for the discriminate binding to the glutamate receptor subtypes prefering the AMPA receptor.

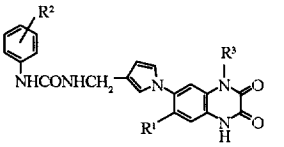
To assess their *in vivo* antagonistic properties the compounds were tested for inhibition of AMPA induced lethal convulsions in mice. In these experiments all compounds were administered intraperitoneally (ip) 60 min. prior to AMPA (40nMol in 10µL H_2O intracerebroventricularly)(see Table 2)[11]. Except for **4a**, all tested pyrrolylquinoxalinediones are superior to NBQX [11,17] and YM90K [5]. The phenylurea **7c** is the most potent compound and its excellent ED_{50} of 0.27mg/kg represents an impressive improvement in potency over NBQX. Furthermore, dicarboxylates **5a**, **5b** and **7b** and monoestercarboxylates **6c** and **6d** were also effective at low dosages (ED_{50} < 5mg/kg). Interestingly, the monocarboxylates **4a** and **7a** missing the methylcarboxylate at the quinoxaline ring exhibited only moderate efficacy indicating some importance of these groups for *in vivo* action.

Table 1: a) Receptor binding with specific radio labelled [^{3}H]-AMPA[14]. The K_i values are mean values for two or more independent experiments. b) K_i value represents result from a single experiment.

	R^1	R^2	R^3	receptor binding [^{3}H]-AMPA K_i(µM)[a]
3a	NO_2	H	H	0.400[11]
b	CF_3	H	H	3.000[11]
c	CF_3	H	CH_2COOH	0.180
d	NO_2	H	CH_2COOH	0.070
4a	NO_2	COOH	H	0.060
b	CF_3	COOH	$CH_2COOCH_2CH_3$	1.500[b]
c	NO_2	COOH	$CH_2COOCH_2CH_3$	0.215
5a	CF_3	COOH	CH_2COOH	0.066
b	NO_2	COOH	CH_2COOH	0.022
6a	CF_3	H	CH_2COOH	0.015[12]
b	CF_3	4-$COOCH_2CH_3$	H	0.450
c	CF_3	3-$COOCH_2CH_3$	CH_2COOH	0.004
d	CF_3	4-$COOCH_2CH_3$	CH_2COOH	0.004
7a	CF_3	4-COOH	H	0.054
b	CF_3	3-COOH	CH_2COOH	0.020
c	CF_3	4-COOH	CH_2COOH	0.007
		NBQX		0.070
		YM90K		0.135



3, 4, 5



6, 7

We also tested the compounds for an efficacy against maximal electroshock seizures (MES) in mice in which compounds were given ip 30 minutes before delivery of the shock. Once more, NBQX revealed a poor efficacy (ED_{50} > 50mg/kg). Except **7a** all pyrrolylquinoxalinediones tested are effective against MES seizures. The dicarboxylates **7c**, **5b** and **5a** display a remarkable efficacy with ED_{50} values of 1.4, 2.4 and 2.4mg/kg, respectively. By contrast, the monocarboxylate **7a** is ineffective at the highest doses tested (ED_{50} >>46mg/kg).

These results raise questions on the importance of the carboxylate residues for the *in vivo* efficacy. Most glutamate antagonists derived from quinoxalinediones, such as CNQX and NBQX, are highly polar compounds and are therefore believed to penetrate poorly the blood brain barrier (BBB) which gives reasonable explanation for their poor *in vivo* efficacy. To cross the BBB compounds use different mechanism such as diffusion or active transports whereas the latter are selective for defined structural moities such as amino acids. A simple approach to estimate the compound's ability to cross the BBB by diffusion is to determine the octanol-water distribution coefficient log Pow and, in general, CNS compounds should have log Pow values between 2 and 4. The dicarboxylates **5** and **7** are also highly polar compounds and, for example, at a pH 7.5 the log Pow's for both **5b** and **7c** are below -3 [19]. Therefore these compounds hardly penetrate the BBB by diffusion. On the other hand, NBQX was suggested to be transported by a carboxylate carrier responsible for both crossing the

	receptor binding			*in-vivo* AMPA antagonism	MES
	[^{3}H]-AMPA[a] K_i (μM)	[^{3}H]-glycine[a] K_i(μM)[b]	[^{3}H]-kainate[a,e] K_i(μM)	ED_{50} (mg/kg) [b]	ED_{50} (mg/kg)[c]
4a	0.060	>30	5.000	17	n.t.[f]
5a	0.066	>30	5.700	2.3	2.4
5b	0.022	>30	5.900	1.4	2.4
6c	0.004	>30	1.200	2.9	28.5
6d	0.004	>30	0.700	0.8	n.t.[f]
7a	0.054	>30	n.t.[f]	≈ 11	46 (0%)[e]
7b	0.020	>30	6.700	3.5	13.5
7c	0.007	>30	3.600	0.27	1.4
NBQX	0.070	33	2.600[h]	> 50[d]	46 (31%)[e,g]
YM90K	0.135	7.4	4.800	13	n.t [f]

Table 2 : a) Affinity constants (K_i-values; means of 2 or more independent experiments) determined by displacement of [^{3}H]AMPA[14] , [^{3}H]glycine[15] or [^{3}H] kainate),[9, 16]. The kainate receptor binding represents binding to high affinity kainate receptors. b) Mean effective dose (ED_{50}), which protects 50% of the mice against AMPA-induced lethality. The compounds were administered ip. 60 minutes prior to application of AMPA intracerebroventricularly (icv.). c) Mean effective dose (ED_{50}) which protects the mice against seizures induced by maximum electroshock via ear electrodes (stimulus parameters: series (duration 0.2 s) of rectangular impulses of 4.64 ms width and 14.7 mA amplitude, frequency 100 Hz). Compounds were given intraperitoneally (ip) 30 min before delivery of shock. d) ED_{50} was 50 mg/kg ip when administered 15 min before AMPA. e) Dose (mg/kg ip) and (% protection). f) not tested g) ED_{50} was 37.6 mg/kg ip when given 5 min before delivery of shock[18]. h) K_i value represents result from a single experiment.

BBB as well as the hepatic uptake from blood [20]. Blocking the carrier by probenecid results in a prolonged *in vivo* activity [18, 21]. Consequently, we assumed that the excellent *in vivo* results of the dicarboxylates may result from an improved penetration of the BBB caused by an active transport[22]. Both carboxylates or carboxylate derivatives are essential residues at the pyrrol ring to be recognized by the carrier and transported effectively. The comparison of the corresponding derivatives **3d** (*in vivo* AMPA antagonism: ED_{50} = 30mg/kg[11]) and **5b** (ED_{50} = 1.4mg/kg) as well as **6a** (ED_{50} = 18mg/kg [12]) and **7c** (ED_{50} = 0.27mg/kg) may support this postulation. The second carboxyl group in **5b** which definitely decreases diffusion through membranes by increasing polarity of a highly polar molecule enhances only slightly the AMPA receptor binding whereas the efficacy in the *in vivo* AMPA antagonism is enhanced 20fold. Likewise, the ureas **6a** and **7c** have roughly comparable AMPA binding but, remarkably, **7c** is >60fold more potent *in vivo*. Missing one or both carboxylates diminish active transport and consequently an *in vivo* activity (see examples **4a** and **7a** or the unsubstituted pyrrols[11]). Conclusively, the exceptional high polarity of the dicarboxylates, the requirement of polar structural moieties (eg. carboxylates), and their location in ligand structure (compare the isomers **7b** and **7c**)[12] suggest a specific carrier which may play a pivotal role for the *in vivo* efficacy of the quinoxalinediones. Nevertheless, further experiments are necessary to verify the influence of an active transport and to identify the nature of the carrier.

One major goal of this work was to provide potent antagonists that have sufficient water solubility. We expected that the quinoxalinediones carrying carboxylate groups have an increased solubility by either mono- or bissalts formation with bases. The solubilities of the compounds **3**-**7** were assessed and, indeed, particulary the dicarboxylates show considerable solubility which enable us to prepare suitable infusion solutions. For example, the dicarboxylates **7c** and **5b** show solubilities >1% in water depending on the employed bases and the adjusted pH value. To prepare 1% solutions the following principle procedure is used: 100mg **7c** were dissolved in 7 ml 0.1M NaOH and the resulting solution was buffered with 0.1M HCl and diluted with H_2O up to the final concentration. According to this procedure solutions are available with pH values ≥5 for **7c** and ≥6.5 for **5b**. Surprisingly, both compounds disclose considerable solubility even in slightly acidic solutions which is not due to salt formation. Alternatively, we prepared Tris-salts from the carboxylates which are known to have improved solubility and, indeed, the bis-Tris-salt of **7c** allows us to provide even 10% water solutions within the pH-range from 6 to 8. Altogether, the solubility in such a range of pH values may be an important benefit since several glutamate antagonists, such as NBQX which is only soluble in alkaline water solutions, have caused serious problems since they precipitate from buffered solutions (pH 6-8) or more fatally in blood [23].

In summary, we synthesized pyrrolylquinoxalinediones carrying a carboxylate group at both the quinoxalinedione ring and pyrrole side-chain. Several compounds were characterized as potent and selective AMPA antagonists in binding assays. Particularly the dicarboxylates, such as **7c**, display a remarkable efficacy in the protection against AMPA induced lethal convulsions and maximal electroshock seizures (MES) in mice.

Moreover, these dicarboxylates allow increased water solubility by bissalt-formation. For example, **7c** converted into a disodium salt has a solubility >1% in water at pH 7.4 . Taken together, these compounds represent suitable candidates to be examined on utility in stroke therapy.

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