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Pyrrolylquinoxalinediones : The importance of pyrrolic substitution on AMPA receptor binding

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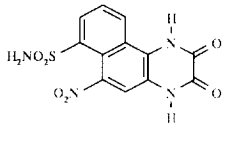
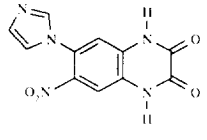
Abstract: Pyrrolylquinoxalinediones were synthesized to elucidate the value of pyrrolic substitution pattern on AMPA receptor binding. We discovered that amide and urea residues at the 3´-position at pyrrol ring favor affinity to AMPA receptor. Particularly, the phenylurea **13n** exhibited a K_i value of 4 nM in AMPA receptor binding and represents the most potent competitive AMPA antagonist reported to date.

The ionotropic AMPA receptor represents one member of the glutamate receptor family[1] and has been proposed to be excessively activated in pathophysiological conditions such as cerebral ischemia and epilepsy [2]. Therefore, antagonists of the AMPA receptor have been suggested for therapy of stroke and epilepsy[3]. Several years ago the discovery of NBQX **1** gave impetus to the search for competitive AMPA antagonists. NBQX was the first AMPA antagonist displaying neuroprotective efficacy in experimental stroke and epilepsy models[4]. During the past years a number of distinct antagonists such as YM90K [5], NS 257 [6], LU 293558 [7], PNQX [8], S17625 [9] and the first noncompetitive antagonist GYKI 52466 [10] were reported, though only few of them (eg. YM90K)[5b] have entered clinical development.

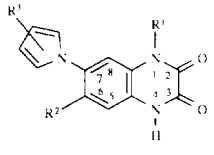
Recently, we presented the pyrrolylquinoxaline-2,3-diones as new AMPA antagonists [11]. In the course of our research program we elucidated the value of the pyrrolic substitution pattern on receptor binding; some results are shown below.



NBQX 1



YM 90K 2



Pyrrolylquinoxalinediones

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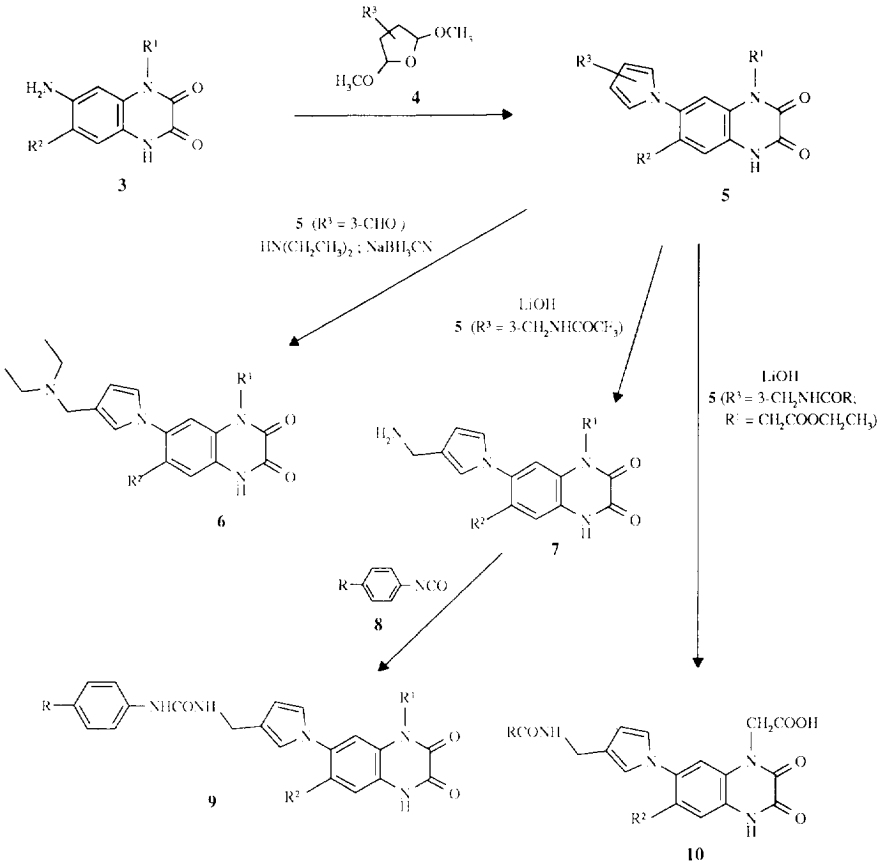
Scheme 1 :



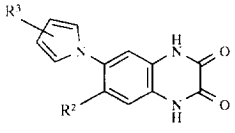
General routes for the synthesis of substituted pyrrolylquinoxalinediones are outlined in Scheme 1.

7-Aminoquinoxalinediones **3** [11] were converted into pyrrols **5** by using a Paal-Knorr reaction procedure. Anilines **3** and appropriate 1,4-dicarbonyl compounds were heated in acetic acid up to 100-120°C. The reaction time, however, had to be limited to 10-30 minutes to avoid decomposition. To synthesize quinoxalinediones carrying carboxylates in R^1, simple ethyl esters **3** ($R^1 = CH_2COOCH_2CH_3$) were employed as protecting groups. These ester groups improved the solubility in organic solvents which was useful for the experimental handling and subsequent chemistry of the intermediates. Indeed, anilines **3** and amines **7** carrying carboxylates in R^1 showed only poor solubility in organic solvents, even dimethylformamide. 2,5-dimethoxytetrahydrofurans **4** were prepared in 3-steps from 3,4-dihydrofuran[12]. The 2,5-dimethylpyrrols **11b**, **12b** and **13b** are available by the above Paal-Knorr procedure using hexane-2,6-dione instead of furans **4**.

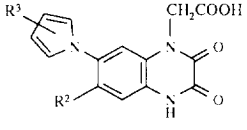
Amides **4** ($R^3 = CH_2NHCOR$) were prepared from amines **4** ($R^3 = CH_2NH_2$)[12] and either acid anhydrides or acid chlorides by convenient methods. Reductive amination of aldehydes **5** (R^3 = CHO) with amines such as diethylamine and $NaBH_3CN$ provided the aminomethylpyrrols **6**. Otherwise, primary amines **7** were synthesised from the trifluoroacetamide **5** ($R^3 = CH_2NHCOCF_3$) by hydrolysis with LiOH. If **5** carried an ester group in R^1 this was also hydrolysed and an aminocarboxylate **7** ($R^1 = CH_2COOH$) was obtained in one step. These aminocarboxylates **7** were added to phenylisocyanates **8** at 80-120°C in dimethylformamide to produce the ureas **9** in good yield. The esters of the oximes **13j** and **14c** were generated by condensation of their aldehydes (R^3 = 3-CHO) with benzylhydroxylamine in ethanol/water mixture at 80°C.

Table 1: a) Receptor binding with specific radio labelled [^{3}H]-AMPA[13]. The K_i values are mean values for two or more independent experiments b) K_i value represents result from a single experiment.

	R^2	R^3	Receptor binding [^{3}H]-AMPA (K_i/µM [a])
11a	CF_3	H	3.000[b]
b	CF_3	2,5-$(CH_3)_2$	>25.000[b]
c	CF_3	2-$COOCH_3$	>25.000[b]
d	CF_3	2-COOH	>25.000[b]
e	CF_3	3-CHO	0.650
f	CF_3	3-CH_2NH_2	0.980
g	CF_3	2-$CONHCH_2Ph$	>25.000
12a	NO_2	H	0.400
b	NO_2	2,5-$(CH_3)_2$	20.000[b]
c	NO_2	3-CH_2NH_2	2.000[b]
d	NO_2	3-$CH_2N(CH_3)CH_2Ph$	0.880
e	NO_2	3-$CH_2NHCONHPh$	0.250
f	NO_2	3-$CH_2NHCONH$-4-pyridyl	0.110
13a	CF_3	H	0.180
b	CF_3	2,5-$(CH_3)_2$	2.000
c	CF_3	3-CH_2NH_2	10.000[b]
d	CF_3	3-$CH_2NHCOCH_3$	0.076
e	CF_3	3-$CH_2NHCOPh$	0.078
f	CF_3	3-$CH_2N(CH_2CH_3)_2$	0.890
g	CF_3	3-$CH_2NHCOCH_2Ph$	0.140
h	CF_3	3-$CH_2NHCONHPh$	0.015
i	CF_3	3-$CH_2NHCONHCH_2Ph$	0.049
j	CF_3	3-C=N-OCH_2Ph	0.250
k	CF_3	3-$CH_2NHCONH$-(4-NO_2-Ph)	0.005
m	CF_3	3-$CH_2NHCONH$-(4-CF_3-Ph)	0.005
n	CF_3	3-$CH_2NHCONH$-(4-Br-Ph)	0.004
14a	NO_2	H	0.070
b	NO_2	3-CH_2NH_2	0.840
c	NO_2	3-CH=N-OCH_2Ph	0.210
d	NO_2	3-CH_2NHCO-4-pyridyl	0.280
e	NO_2	3-$CH_2NHCONHPh$	0.071
f	NO_2	3-CH_2-1-piperidinoyl	3.000[b]
g	NO_2	3-$CH_2NHCOCH_2CH_2Ph$	0.500[b]
h	NO_2	3-$CH_2NHCONHCH_2CH_2$-4-pyridyl	0.095
		NBQX	0.070
		YM90K	0.135



11, 12



13, 14

Except for the ureas **9**, all esters were hydrolyzed to carboxylates (R^1 = CH_2COOH) by LiOH in THF/H_2O mixtures at ambient temperature in the final step.

AMPA receptor affinities were determined by a binding assay described by T. Honoré *et al.*[13] with slight modifications. The results are shown in Table 1.

The unsubstituted pyrrols **11a**, **12a**, **13a** and **14a** were chosen as the starting point for optimization because nitro and trifluoromethyl residues as R^2 and methylcarboxylates as R^1 were reported to favor AMPA receptor binding[5a, 11, 14].

2,5-Dimethyl-substitution at the pyrrol-ring (**11b**, **12b** and **13b**) caused a dramatic drop of affinity to AMPA receptor. Likewise, an ester (**11c**) and a carboxylate (**11d**) in the 2-position were devoid of receptor binding.

Fortunately, substitution in the 3-position of the pyrrol-ring offered more opportunities for optimization. An aldehyde group (**11e**) as well as the aminomethylene group (**11f**) enhanced potency by a factor of 4 and 2 compared to **11a**, respectively. In contrast, the aminomethylene derivative **13c** carrying a carboxylate in R^1 displayed only weak potency (K_i = 10µM) and also within the nitro sets (R^2 = NO_2) the aminomethylene moiety (**12c and 14b**) diminished receptor affinity when compared to the corresponding unsubstituted molecule. Other basic residues in the 3-position such as benzylmethylamine **12d** and diethylamine **13f** did not show any advantages.

On the other hand, incorporation of amide and urea moieties in the pyrrolic side chain has given access to highly potent AMPA antagonists. Both the acetamide **13d** and the benzamide **13e** were 2-fold more potent than **13a** and equipotent to NBQX. Replacement of the amide residue by phenylurea (eg. **13h**;K_i = 15nM) resulted in up to 10 fold more potent derivatives compared with **13a**. Furthermore, substitution in the para-position at the aromatic ring by nitro, trifluoromethyl or bromo favored receptor binding (see compounds **13k**, **13m** and **13n** with their K_i´s of 5, 5 and 4 nM, respectively). These compounds belong to the most potent AMPA antagonists reported to date. For example, **13n** is 17-fold and 31-fold more potent than NBQX **1** and YM90K **2**, respectively. Prolongation of the bridge between aromatic ring and pyrrol-ring by a methylene group to the benzyl urea **13i** offered no advantages.

In contrast, within the nitro sets **12** and **14** the amide and urea residues do not have corresponding effects on affinity.

None of the substituted pyrrolylquinoxalinediones exhibited binding to the glycine binding side at the NMDA receptor (Table 2) while several compounds showed moderate or good affinity to the high affinity Kainate binding side. Indeed, the ureas **13k**, **13m** and **13n** were potent ligands of the Kainate receptors (K_i ≈ 70-110nM). However, selectivity of these compounds for AMPA receptor were still 14-fold or more. The phenylurea **13h** displayed high selectivity versus Kainate binding (> 220fold) and represents a potent and selective AMPA antagonist (K_i = 15nM).

	AMPA[a] K_i/μM	Glycine[a] K_i/μM	Kainate[a)c] K_i/μM	*in-vivo* AMPA antagonism[b] ED_{50} (mg/kg)[c]
12e	0.250	>30[f]	2.790	≈ 30
13d	0.076	>30[f]	4.800	10
13e	0.078	>30[f]	3.300	>30
13h	0.015	>30[f]	3.400	18
13i	0.049	>30[f]	1.670	≈ 30
13k	0.005	>30[f]	0.070	5.5
13m	0.005	>30[f]	0.110	14
13n	0.004	>30[f]	0.110	4.1
14e	0.071	>30[f]	2.600	>30
NBQX	0.070	33[f]	2.600	> 50[d]

Table 2 : a) Receptor binding with specific radio labelled ligands [^{3}H]AMPA[13], [^{3}H]-glycine [15] or [^{3}H]-Kainate [16]. The affinity constants K_i are mean values of two or more independent experiments. b) Intraperitoneal (ip.) dosis of the compound which protected 50% of the animals. c) The compounds were administered ip. 60 minutes prior to application of AMPA intracerebroventricularly (icv.). d) The ED_{50} was 50mg/kg when NBQX was administered 15 minutes prior to AMPA. e) High affinity Kainate binding assay [16]. f) K_i value represents result from a single experiment.

To assess the AMPA antagonistic properties *in vivo*, the compounds were tested for their ability to antagonize AMPA induced lethal convulsions in mice (see Table 2). Each compound was administered intraperitonealy (ip.) 60 min. prior to 40 nmol AMPA (dissolved in 10μL water) being injected intracerebroventricularly and the ED_{50} value was calculated as the dose which protected 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 the blood brain barrier.

In this model NBQX **1** revealed poor efficacy and an ED_{50} of 50mg/kg was only achieved when administered 15 instead of 60 minutes prior to AMPA. This result corresponds with the proposed short half-life of NBQX *in vivo* [17]. Several of the pyrrolylquinoxalinediones showed advantages over NBQX, particularly the carboxylates **13** and **14**. Acetamide **13d** as well as the phenylureas **13h**, **13k**, **13m** and **13n** were potent AMPA antagonists *in vivo* showing ED_{50}'s below 20mg/kg. The nitro-derivative **13k** and the bromo-derivative **13n** represent the most potent compounds with ED_{50}'s of 5.5 and 4.1 mg/kg, respectively. Furthermore, the nitro compound **13k** was effective when administered 15 (ED_{50} = 7mg/kg ip.) as well as 60 minutes prior to AMPA. Therefore **13k** is believed to have a prolonged time of action compared to NBQX and in addition the low effective dosages suggest high blood brain penetration. In conclusion, **13n** and **13k** exhibited superior properties compared to NBQX *in vitro* and *in vivo* and may belong to the most potent competitive AMPA antagonists reported to date.

In summary, we synthesized pyrrolylquinoxalinediones carrying side chains at the pyrrol-ring as new competitive AMPA antagonists. The urea derivatives **13k**, **13m** and **13n** exhibited high AMPA receptor affinity, particularly **13n** is 17-fold more potent than NBQX **1** and 31-fold more potent than YM90K **2**.

Furthermore, the urea **13k** and **13n** revealed good efficacy *in vivo*, inhibiting AMPA induced lethal convulsions at low mg/kg dosages.

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