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Pyrrolylquinoxalinediones Carrying a Piperazine Residue Represent Highly Potent and Selective Ligands to the Homomeric Kainate Receptor GluR5

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Abstract—Pyrrolylquinoxalinediones carrying aminoalkyl residues were evaluated for affinity to the recombinant, homomeric kainate receptors GluR5, GluR6 and GluR7. Most derivatives preferred binding to GluR5. In particular, the piperazine **6e** represents a highly potent and selective antagonist to GluR5.

Glutamate plays a pivotal role as the major excitatory amino acid throughout the central nervous system (CNS) and is involved in many physiological processes such as learning and memory.[1] It is generally accepted that excessive stimulation of cation influx by glutamate causes cell damage in pathophysiological conditions, such as cerebral ischemia, head injury and epilepsy. Therefore, the blocking of selected glutamate receptors is considered to be a promising target in drug research.[2]

The ionotropic glutamate receptor family is divided into three subclasses named after the preferred binding site of the agonists *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) and kainate (Ka).[3] Each of the glutamate receptor subclasses encompasses several subtypes, all of which consist of a combination of distinct subunits. Five kainate subunits, Kai-1, Kai-2, GluR5, GluR6 and GluR7,[4] have been identified. However, there is no reliable evidence as to which subunit combinations may form the native kainate receptors in the brain although some findings suggest defined combinations.[5] In the last decade, the research directed at the ionotropic glutamate family focused on the NMDA and AMPA receptors, and revealed considerable insights into their physiological role.[6] By contrast, kainate receptors and their role in the brain are much less well understood and, for a long time, progress was hampered by a lack of selective antagonists.[7] Meanwhile, a few compounds have been reported, such as LY 377770 **1**[8] and LY 382884 **2**[9] and NS 102 **3**,[10] which display moderate affinity and some selective activity to either GluR5 or GluR6. Nevertheless, there is still a need for more suitable kainate antagonists with improved potency and selectivity.

Previously, we reported the pyrrolylquinoxalinediones as a new class of AMPA receptor antagonists.[11] Among them we noticed several compounds such as the urea derivative **4**, which disclosed high affinity to the native kainate receptor. This finding prompted us to perform screening on a library of pyrrolylquinoxalinedione derivatives for their affinity to the homomeric kainate receptors GluR5, GluR6 and GluR7. Finally, we discovered highly potent ligands, in particular, the 7-(3(4-benzylpiperazinemethyl)-pyrrol-1-yl)-1-carboxymethyl-6-nitro-quinoxaline-2,3-dione **6e** which represents a highly potent and selective antagonist to the homomeric kainate receptor GluR5.

The pyrrolylquinoxalinediones presented herein were either reported earlier or were prepared by routes which we reported previously, such as reductive amination of the corresponding pyrrolic aldehydes.[11] The recombinant human kainate subunits Kai-2,[12] GluR5,[13] GluR6[14] and GluR7[15] were cloned, transfected and stably expressed in cultured human embryonic kidney cells (HEK 293). The affinities of all target compounds to these kainate subunits were evaluated in a convenient displacement assay using [^{3}H]kainate as the radioligand (see Table 1).

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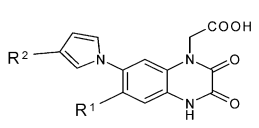
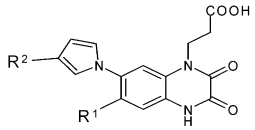
Table 1. The affinities of all target compounds to the recombinant human kainate subunits GluR5, GluR6, GluR7 and Kai-2 were evaluated by a convenient kainate displacement assay using [3]H-kainate as the radioligand



4, **5**, **6**, **8**



7, **9**

	R^1	R^2	K_i (nM)[a]			
			GluR5	GluR6	GluR7	Kai-2
4	CF_3	4-O_2N–C_6H_4–NHCONH–CH_2	100	610	46	12,000
5	NO_2	HOOC	1400	5800	4100	11,000
6a	NO_2	H_2NCH_2	230	1500	nt[b]	9800
6b	NO_2	1-Piperidinyl–CH_2	330	28,000[c]	5700	>30,000
6c	NO_2	4-Ph-1-piperidinyl–CH_2	230	3600	530	18,000[c]
6d	NO_2	$PhCH_2CH_2CH_2NHCH_2$	320	6200	1400	20,000
6e	NO_2	4-Benzyl-1-piperazinyl–CH_2	3.8	4100	680	20,000
6f	NO_2	4-(2-Phenylethyl)-1-piperazinyl–CH_2	12	1400	1400	25,000
7	NO_2	*N*-Benzyl-*N*-methylamine–CH_2	430	23,000[c]	nt[b]	>30,000
8	NO_2	H	530	3900	nt[b]	1300
9	NO_2	H	2400	1200	2500	25,000[c]

The affinity constants K_i are mean values for two or more independent experiments.
[a]Affinity constant.
[b]Not tested.
[c]K_i from one experiment.

As reported earlier, the urea derivative **4** exhibited potent binding to both the native AMPA receptor ($K_i = 5$ nM) and the high affinity kainate binding site at native kainate receptors ($K_i = 70$ nM).[11b] Additionally, **4** disclosed moderate to high affinity to the homomeric kainate receptors GluR5, GluR6 and GluR7 with K_is of 100, 610 and 46 nM, respectively. The unsubstituted pyrrol derivative **8** displayed high affinity to the native AMPA receptor ($K_i = 70$ nM)[11b] and moderate affinity to GluR5 ($K_i = 530$ nM). The homologous pyrrol **9** which carries an carboxyethyl at the quinoxaline moiety exhibited only poor affinity to all kainate receptor subunits. Another derivative, the pyrrolic carboxylate **5**, was found to be a selective AMPA antagonist (AMPA, glycine-site NMDA and Kainate: K_is = 22, >30,000, >5000 nM, respectively).[11c] Unlike **4**, **5** exhibited only poor affinity to GluR5, GluR6 and GluR7 (K_is > 1 μM, see Table 1), which corroborates the proposed selectivity to the AMPA receptor.

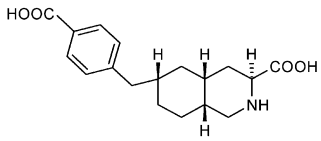
By contrast, insertion of an aliphatic amino group into the pyrrolic site chain attenuated affinity to the AMPA receptor[11b] but retained or even improved affinity to the homomeric kainate receptors.

All derivatives carrying a primary amino group (**6a**), a piperidine (**6b**), a phenylpropylamine (**6d**), a 4-phenylpiperidine (**6c**) or a benzylamine (**7**) disclosed moderate binding to GluR5 (with K_is ranging from 200 to 500 nM). While most of these additionally exhibited binding to either GluR6 or GluR7, the benzylamine **7** and the piperidine **6b** may attract notice due to their good selectivity versus GluR6. However, in comparison to the other derivatives, **7** bears a prolonged carboxylate site chain at the quinoxaline moiety, which may have an impact on the observed discrimination between GluR5 and GluR6. However, the finding that another ethyl carboxylate derivative **9** had only slightly higher affinity in comparison to the corresponding methyl carboxylate **8** is contradictory to this assumption. Nevertheless, all five derivatives (**6a**, **6b**, **6d** and **7**) tended to prefer binding to GluR5.

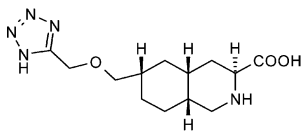
Regarding affinity to GluR5, a breakthrough was achieved by incorporating piperazines into the pyrrolic site chain. The benzylpiperazine **6e** had a K_i of 3.8 nM for GluR5, which means this substance is roughly two orders of magnitude more potent than the monoamines. Furthermore, **6e** showed more than 100-fold selectivity versus GluR6, GluR7 and Kai. Likewise, the homologous phenethyl derivative **6f** exhibited high affinity to GluR5 ($K_i = 12$ nM) and more than 100-fold selectivity over the other kainate subunits.

All **6** and **7** aminoalkyl derivatives exhibited attenuated affinity to the native ionotropic glutamate receptors compared to **4**, **5** or **8**.[11a,b] For example, the piperazine **6e** exhibited no binding to the NMDA glycine site (K_i >30,000 nM), poor binding to the high affinity kainate binding site ($K_i = 5200$ nM) and moderate binding to the AMPA receptor ($K_i = 250$ nM). The amine **7** disclosed only negligible affinity to all three native receptors (NMDA glycine site, AMPA, Ka: all K_is > 23,000 nM) and this will mean **7** exhibited good selectivity to recombinant human kainate receptors.

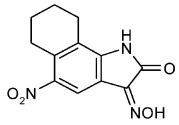
These findings indicate crucial variations in the binding pockets at the AMPA receptor and kainate receptors GluR5, GluR6 and GluR7 with respect to the binding region close to the pyrrol ring.



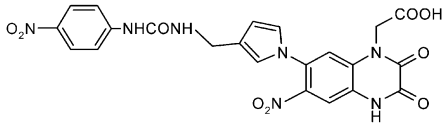
LY382884 **1**



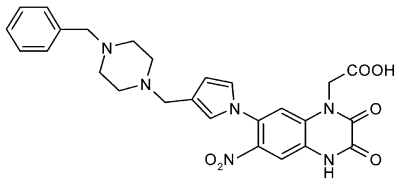
LY377770 **2**



NS102 **3**



4



6e

Pyrrolylquinoxalinediones which carry carbonyl groups within the pyrrolic site chain represent potent AMPA antagonists, but the nature of the carbonyl group controls selectivity versus NMDA and kainite.[11] Interestingly, the urea residue in **4** is tolerated by GluR5, GluR7 and AMPA receptors resulting in potent binding. By contrast, employing the carboxylate moiety as a carbonyl group surrogate at the pyrrol group, such as in **5**, resulted in a drop in affinity to the homomeric kainate receptors, thereby disclosing some variation in the nature of this cleft at the kainate receptors.

The insertion of aliphatic amine residues into the pyrrolic side chain revealed considerable differences with respect to the binding pockets at the kainate receptor subunits.

All of the pyrrolic alkyl monoamines, **6a**, **6b**, **6c** and **7**, exhibited comparable affinity to GluR5 and were roughly as potent as the non-substituted pyrrol **8**. Therefore, the amino residue adjacent to the pyrrol ring seems not to contribute to the affinity for GluR5. On the other hand, in comparison to the urea **4**, the aminomethylene groups are less well tolerated by the kainate subunits GluR6 and GluR7, which indicates that the aminomethylene groups may be an appropriate moiety for increasing selectivity within the kainate receptors. These conclusions are supported by the benzylmethylamine **7**, which exhibited selective affinity to GluR5.

Furthermore, an aminomethylene moiety at the pyrrol ring was less well tolerated by the native AMPA receptor which is indicated by a moderate to substantial decline in affinity in comparison to the unsubstituted pyrrol **8** (**6a**, **6b**, **6c**, **8**: $K_i = 835$, 3080, 420, 25,000 and 70 nM, respectively) and even more so compared to **4** and **5**.[11b]

The strongly enhanced affinity to GluR5 exerted by the piperazines (see **6e** and **6f**) may be attributed to the remote amino group in the piperazine ring. Therefore, this amino group probably forms additional hydrogen bonds with the amino acid residues of this receptor. This type of interaction seems to be restricted to GluR5, which is suggested by the low affinities of **6e** and **6f** for GluR6 and GluR7. Thus, this finding also reveals differences at the binding pockets of the homomeric kainate receptor subunits located at the cleft at which the pyrrolic ring is directed.

Finally, the distal aromatic rings at the pyrrolic site chains might also play an important role in improving affinity and selectivity. All compounds, which showed high affinity to GluR5, carry a distal aromatic ring at the pyrrolic site chain. But a potential contribution to GluR5 affinity by the distal aromatic rings may be questionable since no beneficial effect was observed in the monoamine set (see examples **6c**, **6d** and **7**).

Based on these results, we assume that the amino group adjacent to the pyrrolic ring may prove to be useful in enhancing selectivity and affinity to. Both findings may have an impact on designing ligands with superior properties of potency and selectivity to the kainate receptor subtypes.

6e, **6f** and **7** were tested for efficacy against NMDA and AMPA induced convulsions in mice, which were models we used to gain data on brain penetration and in vivo efficacy.[11] The three compounds protected the animals from the induced convulsions in a dose-dependent manner. For example, the ED_{50}s were 14, 21 and 3.3 mg/kg for **6e**, **6f** and **7**, respectively, when given intraperitoneally 60 min prior to inducing the convulsions by NMDA. Therefore, the three compounds penetrate into the brain and may represent antagonists due to their antiglutaminergic efficacy. Furthermore, when transformed into the appropriate salts, the three compounds displayed considerable water solubility, a property which allows for intravenous administration.

Both amines **6e** and **6f** represent selective antagonists to GluR5. But there is no evidence that homomeric GluR5 receptors are present in the brain although kainate receptors in the dorsal root ganglion are reported to disclose related electrophysiological characteristics.[16] However, GluR5 assembles with other kainate subunits to form heteromeric kainate receptors.[17] Indeed, such an ion gating heteromeric receptor has been formed by

coexpression of rKai-1 and rGluR7[18] and, furthermore, antibody labeling of kainate subtypes confirms the existence of heteromeres in the brain.[19] In the brain itself, [^{3}H]kainate labels two populations of binding sites: high and low affinity binding sites.[20] The affinity of a range of kainate ligands suggests that the low affinity kainate binding site may be related to GluR5, GluR6 and GluR7.[5,17] The novel ligands presented here preferred binding to GluR5 and therefore represent suitable tools for elucidating the role of the distinct kainate receptors in brain.

In conclusion, we discovered several compounds with high affinity to the homomeric recombinant kainate receptor GluR5. The pyrrolylquinoxalines **6e** (BSF 91594), **6f** (BSF 111886) and **7** (BSF 84077) may represent selective antagonists to GluR5. Moreover, **6e** represents one of the most potent antagonists to GluR5 reported to date.

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