Pergamon

Bioorganic & Medicinal Chemistry Letters 10 (2000) 1807–1810

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

4-Alkylidenyl Glutamic Acids, Potent and Selective GluR5 Agonists

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Received 27 March 2000; revised 13 June 2000; accepted 14 June 2000

Abstract—Twenty-four 4-alkylidene glutamic acids were synthesised and tested as potential subtype selective GluR5 and 6 ligands. It was found that a critical size of alkylidene group gave potent and selective GluR5 receptor agonists. LY339624 had K_is of 0.0326 and >100 μM on GluR5 and 6 receptors, respectively. © 2000 Published by Elsevier Science Ltd. All rights reserved.

L-Glutamate (Glu) is the major excitatory neurotransmitter in the mammalian central nervous system (CNS). Glutamate receptors are subdivided into ionotropic (GluRs)[1,2] and metabotropic receptors (mGluRs).[3,4] The ionotropic receptors mediate fast synaptic transmission through ligand-gated ion channels. These ionotropic receptors are subdivided into three classes on the basis of their sensitivity to NMDA (*N*-methyl-D-aspartate), KA (kainate) and AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate), from which they take their names.[5] Studies have shown there are five distinct kainate receptor subunits: GluR5, GluR6, GluR7, KA1, and KA2 (for a review see ref 6). GluR5, 6 and 7, when expressed homomerically, form functional ion channels activated by kainate, domoate or glutamate.[7,8] Since these individual kainate binding proteins have different distributions within the CNS,[9,10] subtype selective kainate ligands may be expected to show distinct pharmacology and may thus have therapeutic potential. Unfortunately, there are very few kainate subtype selective ligands, hence the therapeutic potential of such agents has not been realised. In an earlier paper, we reported that the 4-substituted glutamate analogue LY339434 is a potent and selective GluR5 receptor agonist (K_i 14.8 nM).[11] This paper extends the structure–activity relationship (SAR) to a number of alkylidene glutamate analogues (Fig. 1), some of which are potent and selective GluR5 receptor agonists. This study also gives insight into the differences in the receptor binding sites of GluR5 and 6.

Chemistry

The compounds were prepared as shown in Scheme 1 by $BF_3{\cdot}OEt_2$ mediated aldol condensation of *S*-ethyl pyroglutamate **1** with aldehydes and ketones.[12] The resultant diastereomeric mixtures of the alcohols **2** were dehydrated[13] to give predominately the *E* isomers of the 4-alkylidenepyroglutamates **3** which were purified by flash column chromatography. Hydrolysis of **3** was accomplished with aqueous LiOH in THF to produce the *N*-BOC-protected diacid followed by removal of the BOC group with a saturated solution of HCl in ethyl acetate at room temperature to give the final products **4**.

Biological Results

The compounds were tested in triplicate in a radioligand binding assay, using cell membranes prepared from HEK293 cells expressing the respective human receptor subtypes. Results are expressed as K_i values in μM. In Table 1, we report the effects of linear and branched alkyl groups. The compounds with the smallest groups, e.g., hydrogen (**5**) and methyl (**6**) are equiactive on GluR5

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PII: S0960-894X(00)00346-2

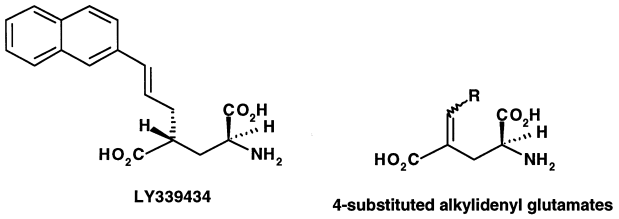
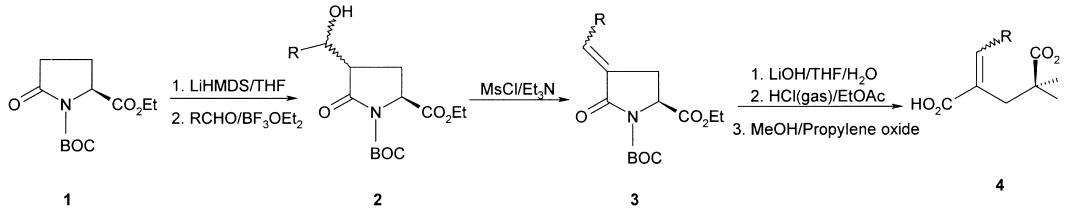


Figure 1.

and 6 receptors. Further increasing the size of the alkyl group greatly reduces the activity at GluR6 and in contrast the affinity at GluR5 increases slightly. The net effect is that compounds with the larger alkyl chains, e.g., *n*-propyl (**8**) and *n*-butyl (**9**) show greater than 700-fold selectivity for GluR5 over GluR6. The introduction of an additional double bond, as in compound **10**, has little effect on activity. Compounds with branched alkyl chains show similar potency to their straight-chain analogues at GluR5, but the activity at GluR6 is further reduced, making these even more selective, with affinity ratios of greater than 1000. These encouraging results with larger alkyl groups led us to investigate the effect of having a cycloalkyl or aryl group either directly bonded to the olefin or attached via a spacer group (Table 2).

Further increasing the size of the substituents on the olefin causes the GluR5 activity to be reduced with only the methyl cyclopentyl compound (**15**) showing good potency. Finally, we investigated the effects of having two substituents on the double bond, as shown in Table 3.

The SAR trend was similar to that seen with the mono-substituted compounds. There was an initial decrease in activity with the methyl compound (**22**) compared with the unsubstituted analogue (**5**) but, as the size of the alkyl group increased, the potency on GluR5 improved,



Scheme 1.

Table 1.

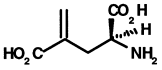
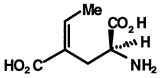
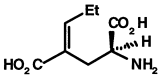
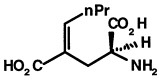
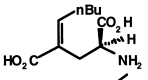
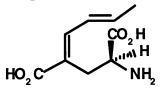
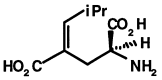
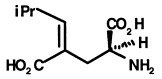
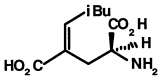
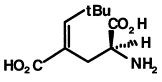
Compound no.	Structure	K_i (μM) GluR5	K_i (μM) GluR6	Ratio GluR6/GluR5
5		0.27	0.45	1.67
6		2.2	2.5	1.14
7 LY310683		0.0605	18.0	298
8		0.0804	57.2	711
9		0.150	125.0	833
10		0.0511	16.3	319
11 LY339637		0.0544	82.5	1517
12 LY339675		0.0936	>500.0	>5342
13		0.224	634.0	2870
14		0.0857	>100.0	>1167

Table 2.

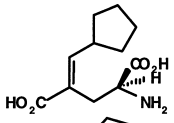
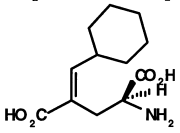
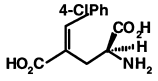
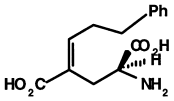
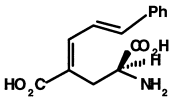
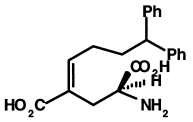
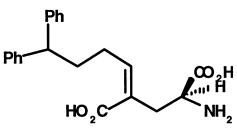
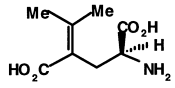
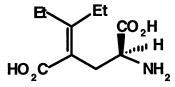
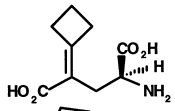
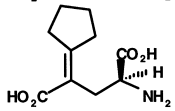
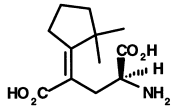
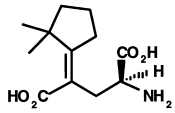
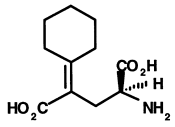
Compound no.	Structure	K_i (μM) GluR5	K_i (μM) GluR6	Ratio GluR6/GluR5
15		0.152	87.6	576
16		6.06	>100.01	>16.5
17		41.2	193.0	4.7
18		17.1	205.0	12.0
19		29.1	>100.0	>3.4
20		>1.0	>100.0	100
21		>1.0	>10.0	10

Table 3.

Compound no.	Structure	K_i (μM) GluR5	K_i (μM) GluR6	Ratio GluR6/GluR5
22		13.9	>400.0	>28.8
23 LY339687		0.241	207.0	859
24		0.470	>100.0	>213
25 LY339624		0.0326	>100.0	>3060
26		0.236	>100.0	>424
27		3.62	46.3	12.8
28		5.13	255.0	49.7

reaching a maximum with the cyclopentyl compound (**25**). The more bulky substituted cyclopentyl compounds **26** and **27**, and the cyclohexyl compound (**28**), were significantly less potent, again demonstrating that there is a clear limit to the size of alkyl group the GluR5 receptor will accept. The most potent and selective compounds were then tested further on the other kainate and AMPA receptors, as shown in Table 4. The most selective GluR5 ligand was the *Z* isopropyl compound LY339675 (**12**) which had a K_i value greater than 10 μM across the kainate and AMPA receptors tested. Disappointingly, the most potent compound LY339624 (**25**) had significant affinity for GluR4 receptor subtype, with a K_i value of 0.533 μM, while the *E* isopropyl compound LY339637 (**11**) had significant affinity at GluR1 and GluR7 receptors. Although none of these compounds was as potent as LY339434, they did show different affinity profiles and, in some cases, better subtype selectivity.

Finally we investigated whether the compounds were agonists or antagonists using patch clamp electrophysiology on the transfected HEK 293 cells, as shown in Table 5.

All the compounds tested were found to be agonists on GluR5 receptors with efficacy similar to or slightly less than kainic acid. LY339624 was also tested on rat dorsal root ganglion (DRG) neurons (thought to express GluR5 receptors) (EC_{50} 1.05 μM) to confirm that the compound was an agonist on a native preparation as well as on the HEK 293 GluR5 construct.

In summary, the GluR5 receptor binding of glutamate is enhanced by the introduction of a lipophilic 4-alkylidene group of a critical size. In contrast, the potency of glutamic acid analogues at GluR6 receptors is reduced by the introduction of a 4-alkylidene group. This important difference could be useful in the design of subtype selective GluR5 receptor ligands.

Experimental

General procedure: synthesis of 4-alkylidene pyroglutamates

The 4-alkylidenepyroglutamates **3** were prepared from protected pyroglutamate as reported earlier[12] and then

Table 4.

Compound	GluR1 K_i (μM)	GluR2 K_i (μM)	GluR4 K_i (μM)	GluR5 K_i (μM)	GluR6 K_i (μM)	KA2 K_i (μM)	GluR7 K_i (μM)
Kainate	7.5±2.0 (8)	12.2±2.7 (4)	1.71±0.17 (3)	0.177±0.022 (3)	0.0324±0.0013 (4)	0.08±0.002 (5)	0.010±0.002 (3)
Glutamate	1.36±0.26 (3)	0.940±0.09 (3)	0.868±0.0219 (3)	0.701±0.046 (3)	1.106±0.02 (3)	0.750±0.077 (3)	0.789±0.083 (3)
LY339434	>10.0	>10.0	>10.0	0.0148	15.41	>10.0	0.617
LY339637	0.70	3.70	5.6	0.0544	82.5	7.50	0.518
LY339675	113.4	44.9	10.3	0.0936	500.0	82.2	10.5
LY339687	>100.0	280.9	4.71	0.241	207.4	21.4	12.4
LY339624	1.10	3.68	0.533	0.0326	>100.0	10.0	5.73

Table 5.

Compound	GluR5		GluR6		DRG cells	
	EC_{50} (μM)	R_{max} (%)	EC_{50} (μM)	R_{max} (%)	EC_{50} (μM)	R_{max} (%)
Kainate	16.2±1.0 (4)	100 (4)	0.7±0.1 (6)	100	12.0±0.1 (4)	100
Glutamate	75.0±7.5 (15)	79±2 (15)	25±2 (8)	n.d.	35.2±0.2 (5)	51±10 (5)
LY339434	2.5±0.86 (3)	80±0.08 (3)	>100 (5)	n.d.	0.8±0.2 (6)	57±5 (6)
LY310683	14.2±1.64 (3)	94±4.0 (4)	>300 (5)	n.d.	n.d.	n.d.
LY339624	6.2±3.8 (4)	79±12.9 (4)	>100	n.e.	1.05	n.d.

deprotected as follows: To a solution of the pyroglutamate **3** (2 mmol) in THF (15 mL) was added a 2.5 N aqueous solution of LiOH (14.4 mL, 36 mmol). The mixture was stirred at room temperature for 4 h and then acidified to pH 2 with 1 N HCl solution and extracted with diethyl ether (3×20 mL). The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo to give an oily residue, which was fully deprotected by treatment with a saturated HCl solution in ethyl acetate for 1 h at room temperature. After evaporation to dryness, the resulting white solid was triturated with diethyl ether. The amino acids **4** were isolated either as hydrochlorides or as zwitterions by treatment of a methanolic solution of the hydrochloride with propylene oxide.

Binding studies and electrophysiology

The binding and electrophysiological studies were performed as described by Clarke et al.[14]

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13. The dehydration was carried out with mesyl chloride and triethylamine except where R = *t*Bu when it was necessary to use Burgess reagent: Burgess, E. M.; Penton, H. R.; Taylor, E. A. *J. Org. Chem*. **1973**, *38*, 26.
14. Clarke, V. R. J.; Ballyk, B. A.; Hoo, K. H.; Mandelzys, A.; Pellizzari, A.; Bath, C. P.; Thomas, J.; Sharpe, E. F.; Davies, C. H.; Ornstein, P. L.; Schoepp, D. D.; Kamboj, R. K.; Collingridge, G. L.; Lodge, D.; Bleakman, D. *Nature* **1997**, *389*, 599.