Articles

Synthesis of a Series of Aryl Kainic Acid Analogs and Evaluation in Cells Stably Expressing the Kainate Receptor humGluR6

Buddy E. Cantrell,[†] Dennis M. Zimmerman,*[,†] James A. Monn,[†] Rajender K. Kamboj,[‡] Ken H. Hoo,[‡] Joseph P. Tizzano,[†] Ian A. Pullar,[§] Louise N. Farrell,[§] and David Bleakman[§]

Eli Lilly and Company, Lilly Research Laboratories, Lilly Corporate Center, Indianapolis, Indiana 46285, Lilly Research Center, Ltd., Erl Wood Manor, Windlesham, England, and Allelix Biopharmaceuticals Inc., Mississauga, Ontario, Canada L4V 1P1

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The synthesis and pharmacological characterization of a novel series of 4-aryl-substituted kainic acid analogs are described. Receptor affinities were determined on recombinantly expressed humGluR6 kainate receptors and on [^{3}H]kainate binding to rat forebrain kainate receptors. Functional agonist potencies were assessed using whole cell voltage clamp recordings in cells expressing humGluR6 receptors. Substitution of phenyl for the methyl at the C-4 position of kainic acid produced **11** which has high affinity and agonist potency at the GluR6 receptor. Substitution on phenyl led to a series of compounds with varying affinity for this kainate receptor. Agonist potency correlated with receptor affinity and with no derivative could antagonist activity be identified. Affinities for the humGluR6 kainate receptor were approximately 10−50 less than the observed affinities at rat forebrain kainate receptors. Furthermore, within the series of 4-aryl-substituted kainic acid analogs, there was a high degree of correlation between binding affinities for humGluR6 receptors and competition with kainate binding to rat forebrain kainate receptors.

Glutamate is the primary excitatory transmitter in the mammalian central nervous system (CNS), and abnormalities in glutamatergic transmission have been implicated in a wide range of CNS disorders. The development of selective ligands for the modulation of the actions of glutamate remains a challenging but promising strategy for therapeutic intervention in these disorders.

Glutamate is known to act at a number of receptors. These have been broadly dividied into two classes: those which form ligand-gated ion channels[1−5] (ionotropic glutamate receptors) and those linked to second-messenger systems via G-proteins[6,7] (metabotropic glutamate receptors). For the ionotropic class of glutamate receptors there are three major types which are defined by the action of the subtype selective agonists *N*-methyl-D-aspartate (NMDA), kainate, and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA).

Molecular cloning has verified the distinction of NMDA, kainate, and AMPA receptors and further showed that they exist as families of receptors. Five kainate receptors have been cloned, expressed, and demonstrated to have high affinity (GluR7, KA1, and KA) or low affinity (GluR5 and GluR6) for kainate.[8−15] When expressed in their homomeric configuration, GluR5 and GluR6 but not GluR7, KA1, or KA2 form functional ion channels activated by the agonists kainate, domoate, or glutamate. Kainate receptors are widely distributed in the CNS,[5] but individual receptor proteins show discrete localization, for example in the hippocampus[5] and spinal cord.[16] Thus, selective kainate receptor ligands are expected to produce distinct pharmacological activities.

Kainic acid and other kainoids such as domoic acid are highly neurotoxic, and their patterns of neurotoxicity and resulting CNS dysfunction have been well characterized.[17−19] These and other findings suggest important potential therapeutic uses of kainate antagonists including the treatment of epilepsy, pain, and acute and chronic neurodegeneration. Very recently presynaptic kainate receptors, which when activated inhibit the release of glutamate, have been identified.[20] Thus, highly selective agonists for these receptors, if not neurotoxic, may also have important therapeutic potential.

Of the ionotropic glutamate receptors, the pharmacology of kainate receptors is the least understood primarily because of the limited number of selective ligands, agonists and antagonists, available for pharmacological studies. In fact, prior to the molecular cloning findings, distinctions between AMPA and kainate receptor-mediated pharmacology were unclear because of kainate's ability to activate AMPA receptors. The recent cloning and expression of these receptors has now identified kainate receptor-mediated pharmacology and led to the development of more clear and efficient strategies for the discovery of selective kainate receptor ligands. Specific functional receptors can now be expressed in mammalian cells. These can be used to determine definitive receptor affinity values and also to provide a direct quantitative measure of agonist and antagonist effects. Selective agonists and antagonists can then be studied *in vitro* and *in vivo* to further understand pharmacological properties of native kainate receptors.

[†] Lilly Corporate Center.
[‡] Allelix Biopharmaceuticals Inc.
[§] Lilly Research Center, Ltd.
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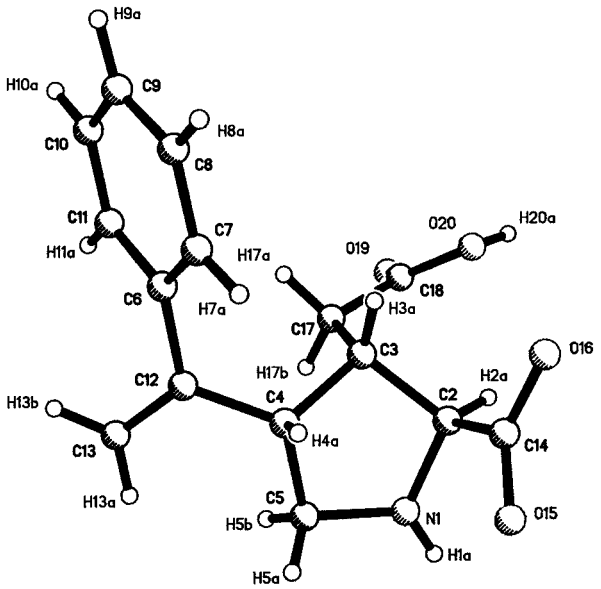
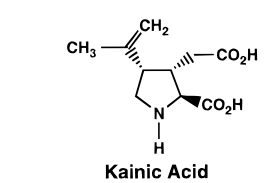


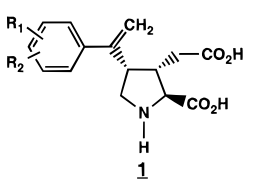
Figure 1. X-ray crystal structure of (2*SR*,3*SR*,4*SR*)-2-carboxy-4-(1-phenylethen-1-yl)pyrrolidine-3-acetic acid (**11**).

In accord with this, the stable expression of the human (hum) GluR6, also termed EAA4, kainate receptor has been reported.[14] This receptor, when expressed in HEK293 cells, possesses kainate- but not AMPA (or NMDA)-evoked functional properties. These cells have been used to determine relative affinity values in ligand binding studies with [^{3}H]kainate and to assess agonist and antagonist potencies using whole cell voltage clamp electrophysiology. These and other findings[20,21] have shown that homomeric expressed GluR6 receptors have properties similar to certain native kainate receptors. Thus, this cloned kainate receptor appears to be highly suited for the characterization of novel kainoid ligands.

In general, structure−activity relationships of kainic acid have only been explored to a limited extent, in part, because of limitations of previously available test systems but also because of the complexity of its structure (Figure 1).[17,22] Kainic acid possess three contiguous stereocenters, and their relative configurations are vital for high affinity to kainate receptors. In addition, affinity for the kainate receptor is markedly reduced with reduction of the vinyl substituent. Recently an efficient synthesis of kainic acid was reported, and the approach appeared suitable for analog synthesis.[23] Structural modification of the C-4 position of kainic acid appeared feasible and, furthermore, warranted because of the high potency of domoate and analogs of acromelic acid for kainate receptors.[24−28] Accordingly, we undertook a structure−activity relationship study of aryl-substituted kainic acid analogs (**1**−**3**). In this study



Kainic Acid



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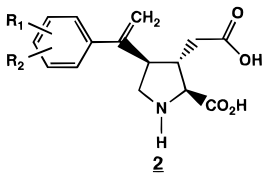

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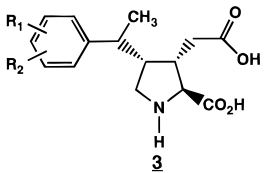

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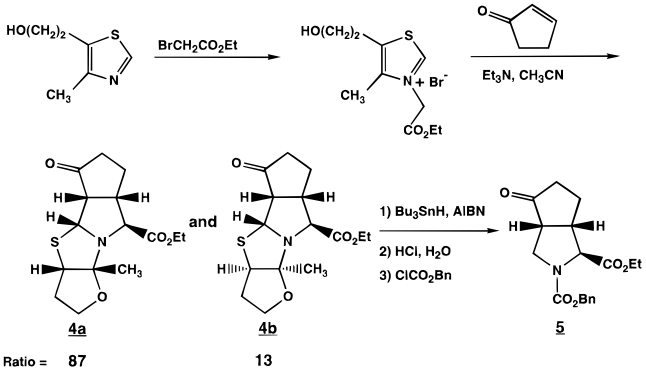
Scheme 1



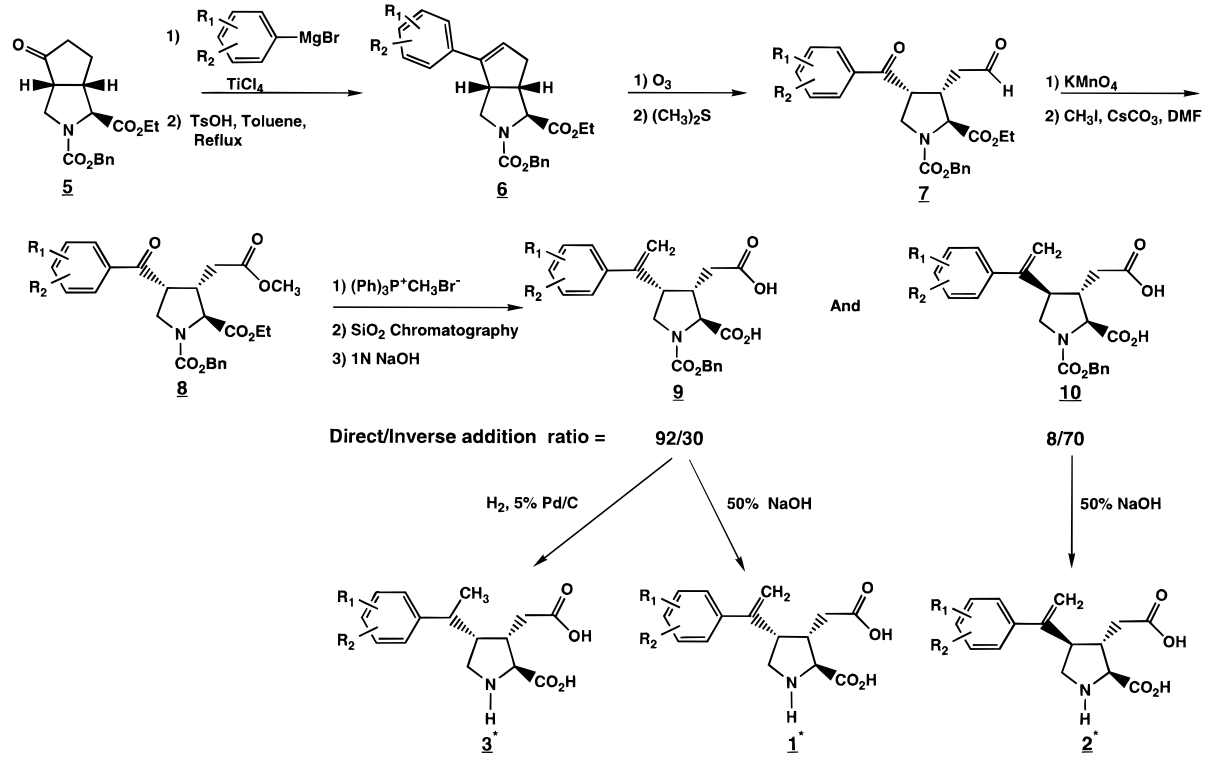
these aryl kainates were evaluated for agonist and antagonist properties in cells stably expressing humGluR6 kainate receptors. Our goal was to characterize structure−activity relationships for both binding and activating humGluR6 receptors and, if possible, discover humGluR6 receptor antagonists. Affinities for rat forebrain kainate receptors were also determined to examine potential structure−activity relationship differences for these potentially different kainate receptors. Our designed strategy was relatively straightforward. The first approach involved the synthesis and evaluation of simple phenyl-substituted derivatives. There is ample precedence for relatively minor structural changes markedly affecting receptor affinities as well as intrinsic activities. In addition, we wanted to add large lipophilic substituents to the kainic acid molecule, remote from its key receptor recognition elements, to explore the feasibility of inhibiting activation of the receptor through additional lipophilic binding interactions.

Chemistry

The synthesis of the central azabicyclooctanone intermediate **5** for the preparation of kainic acid and kainic acid analogs is depicted in Scheme 1. 4-Methyl-5-thiazoleethanol was condensed with ethyl bromoacetate to form the thiazolium bromide salt which, in the presence of triethylamine and 2-cyclopentenone, affords the tetracyclic thiazoline cycloaddition products **4a**,**b** in good (70−80%) isolated yields.[23,29] Reductive cleavage of the thiazoline C−S bond (Bu_3SnH, AIBN) followed by solvolysis of the intermediate hemiaminal (HCl, H_2O) and protection of the secondary amine as its benzyl carbamate ($ClCO_2Bn$, NaOH) afforded the fully protected 2-carboxy-3-azabicyclooctan-6-one (**5**) in 64% overall yield.[23]

Reaction of the one-to-one adduct of $TiCl_4$ and various aromatic Grignard reagents[30] with **5** followed by dehydration produced bicyclic styryl derivatives **6**, Scheme 2, in good (63−94%) isolated yields. Oxidative cleavage of the trisubstituted olefin with ozone fillowed by reductive workup afforded pyrrolidine-3-acetaldehyde derivatives **7** (83−99%) which were converted to the corresponding pyrrolidine-3-acetate methyl esters **8** by buffered permanganate oxidation[31] followed by alkylative esterification (MeI, Cs_2CO_3) (72−94%). Reaction of **8** with methyltriphenylphosphorane afforded the methylenated products **9** and **10** (58−86%) in which varying degrees of epimerization at the C-4 carbon atom were observed. The degree of epimerization observed in the methylenated products was found to be highly dependent on the order of addition of the reactants.

Scheme 2



* Compounds 1, 2, and 3 are racemic mixtures.

Addition of the ketone substrate to the phosphorus ylide (direct addition) afforded a high ratio (92:8) of the desired nonepimerized (kainate-like) methylenated products **9** to the undesired C-4-epimerized (allo kainate-like) products **10**. Conversely, addition of the phosphorus ylide to the ketone substrate (inverse addition) gave rise to product mixtures favoring (70:30 ratios) the allo diastereomer **10**. Methylenated products **9** and **10** were separated chromatographically and subsequently hydrolyzed (45−60%) to yield aryl kainic acid and aryl allo kainic acid derivatives **1** and **2**, respectively. Alternatively, some derivatives of **9** were subjected to catalytic hydrogenation followed by hydrolysis to afford aryl dihydro kainate derivatives **3**.

The relative configurations at C-2, C-3, and C-4 for **11** were determined by X-ray analysis (Figure 1). Confirmation of kainoid versus allo configurations was then obtained through ^{1}H NMR comparisons. The structures and physical chemistry data for the final products synthesized and characterized as racemates are provided in Table 1.

Results

Inhibition constants (K_i's) for the humGluR6 receptor and for binding to rat forebrain kainate receptors for the 4-(α-arylvinyl) kainate analogs are shown in Table 2. Also provided in Table 2 are the potencies of the compounds to evoke inward currents in intact cells expressing humGluR6. For this measure of kainate agonist activity, test compounds were evaluated at concentrations of 1, 10, and 30 μM and the data are presented as percent of the response evoked by the kainate receptor agonist domoate.[27] As shown in Table 2, the α-phenyl derivative **11** has high affinity for the humGluR6 receptor. Its affinity, as a racemic mixture, is equivalent to that of kainic acid and approximately one-half that of domoate. Application of **11** to intact cells evoked inward currents and showed that **11** was a potent agonist. Reduction of the vinyl group of **11**, producing the diastereoisomeric mixture **43**, resulted in a marked loss of receptor affinity (>3000-fold). Inversion of the C-4 stereocenter, compound **39**, reduced receptor affinity approximately 200-fold. As shown in Table 2, these structural changes also caused a similar loss of receptor affinity with phenyl-substituted derivatives **40**−**42** and **44.**

Substitution of methyl (**12** and **13**) or methoxy (**14** and **15**) on the phenyl group of **11** at the 3′ or 4′ position produced only a small loss of humGluR6 receptor affinity, but the loss of activity was greater with the 3′-substituted derivatives. The 4-chloro (**16**) and 4-fluoro (**17**) derivatives, however, have high affinity for the humGluR6 receptor. Disubstitution (**18**−**20**) of phenyl with these groups again produced only minor changes in receptor affinities. Electrophysiological evaluation in intact cells showed that all of these compounds activated humGluR6 receptors and produced a response at a concentration of 10 μM at least 70% that of domoate at 1 μM.

Inspection of Table 2 shows that a wide variety of groups could be substituted for hydrogen at the 3- and 4-phenyl positions. Increasing the size of the alkyl substituent on phenyl in general caused a loss of receptor affinity. The loss of activity with the 4′-*tert*-butyl substituent (**24**) was approximately 10-fold. The 3′-and 4′-phenyl-substituted phenyl derivatives **27** and **28,** however, have high affinity for the humGluR6 receptor and are potent agonists in the whole cell functional assay. In general, substitution of the 3′ and 4′ positions with larger lipophilic substituents decreased affinity for the humGluR6 receptor. There were some notable exceptions, including compounds **30** and **33**. A

Table 1. Structure and Physicochemical Data for 2-Carboxy-4-(1-arylethen-1-yl)pyrrolidine-3-acetic Acids **1**−**3**

compd	structure	R_1	R_2	mp (°C)	recryst solvent
11	**1**	H	H	162.5−164.5	H_2O
12	**1**	3-CH_3	H	>178 dec	H_2O/CH_3CN
13	**1**	4-CH_3	H	>215 dec	H_2O
14	**1**	3-OCH_3	H	>185 dec	H_2O
15	**1**	4-OCH_3	H	>180 dec	H_2O
16	**1**	4-Cl	H	>223 dec	H_2O
17	**1**	4-F	H	>131 dec	
18	**1**	3-CH_3	4-CH_3	>183 dec	H_2O/CH_3CN
19	**1**	3-CH_3	5-CH_3	>198 dec	H_2O/CH_3CN
20	**1**	3-CH_3	4-F	>186 dec	H_2O/CH_3CN
21	**1**	4-CH_2CH_3	H	>170 dec	
22	**1**	4-$CH_2CH_2CH_3$	H	>168 dec	H_2O/CH_3CN
23	**1**	4-$CH_2(CH_2)_2CH_3$	H	>172 dec	H_2O/CH_3CN
24	**1**	4-$C(CH_3)_3$	H	>235 dec	H_2O/CH_3CN
25	**1**	4-$CH(CH_3)=CH_2$	H	>165 dec	H_2O/CH_3CN
26	**1**	4-$C(CH_2CH_3)=CH_2$	H	>169 dec	CH_3OH
27	**1**	3-Ph	H	>191 dec	H_2O/CH_3CN
28	**1**	4-Ph	H	>16 dec	CH_3OH
29	**1**	3-$O(CH_2)_3CH_3$	H	>205 dec	H_2O/CH_3CN
30	**1**	4-$O(CH_2)_3CH_3$	H	>188 dec	H_2O/CH_3CN
31	**1**	4-OPh	H	>203 dec	H_2O/CH_3CN
32	**1**	3-$O(CH_2)_2Ph$	H	>175 dec	H_2O/CH_3CN
33	**1**	4-$O(CH_2)_2Ph$	H	>196 dec	H_2O/CH_3CN
34	**1**	4-$O(CH_2)_5Ph$	H	>203 dec	H_2O/CH_3CN
35	**1**	3-$O(CH_2)_2CH(Ph)_2$	H	>220 dec	H_2O/CH_3CN
36	**1**	3-OCH_3	4-$O(CH_2)_2Ph$	>173 dec	H_2O/CH_3CN
37	**1**	3-CH_3	4-$O(CH_2)_2Ph$	>165 dec	H_2O/CH_3CN
38	**1**	3-CH_3	4-$O(CH_2)_4CH_3$	>213 dec	H_2O/CH_3CN
39	**2**	H	H	>195 dec	H_2O
40	**2**	4-$C(CH_3)=CH_2$	H	>193 dec	CH_3OH
41	**2**	3-Ph	H	>196 dec	H_2O/CH_3CN
42	**2**	3-$O(CH_2)_2CH(Ph)_2$	H	196.5−198	H_2O/CH_3CN
43	**3**	H	H	>260 dec	H_{20}/CH_3CN
44	**3**	3-Ph	H	>166 dec	H_2O/CH_3CN

few disubstituted derivatives with one group being a large lipophilic substituent were synthesized and evaluated, and the high potency of **37** compared to **36** is noteworthy.

An examination of the affinities of the 4-(α-arylvinyl) kainates for rat forebrain kainate receptors (data shown in Table 2) shows that all compounds bound with significantly higher affinity (approximately 10−50-fold) to rat forebrain receptors than to the humGluR6 receptor. Furthermore, the structure−activity relationships observed were essentially identical with those already described for binding to the humGluR6 receptor. Figure 2 shows the correlation between the receptor affinities (log K_i's) of the aryl kainates for the humGluR6 receptor and rat forebrain kainate receptors. A highly significant correlation was observed.

Discussion

Modification of a recently developed synthesis of kainic acid has allowed the efficient synthesis of a wide variety of 4-[α-(substitutedphenyl)vinyl] kainic acid analogs. Several compounds possessing the kainate configuration as racemates (**1**) in this series were found to have high affinity and functional activity for the humGluR6 receptor and also high affinity for rat forebrain kainate receptors. Thus, substitution of aryl for methyl in kainic acid is well tolerated for binding and activating kainate receptors. Reversal of the configuration at C-4 and reduction of the vinyl group markedly reduced receptor affinities. These alterations of kainic acid are also known to cause a similar loss of kainate receptor affinity.[17] Thus, the binding of the 4-(arylvinyl) kainates appears similar to that of kainic acid.

Within this series, the structure−activity relations observed for binding to the humGluR6 receptor and rat forebrain kainate binding were highly correlative even though the affinity for the rat brain receptors was markedly higher. The differences in relative affinities for these receptors appear to indicate differences in the molecular composition between rat forebrain kainate receptors and the humGluR6 receptor. It is known from Scatchard analysis that recombinant rat and human GluR6 receptors have equivalent K_d's for kainic acid. It is also known that the K_d for kainic acid for rat forebrain receptors is very similar to the K_d's derived for kainic acid for recombinantly expressed KA1, KA2, and GluR7 receptors. Thus, the involvement of one or more of these proteins in high-affinity kainate receptor rat forebrain binding is suspected. We, therefore, speculate that the high correlation of binding to the humGluR6 receptor and rat forebrain kainate receptors indicates a common environment for binding of the 4-aryl substituent of the 4-(arylvinyl) kainic acids to different kainate receptors.

Application of the 4-(α-arylvinyl) kainates to cells expressing humGluR6 produced agonist responses, and agonist potency correlated with receptor affinity. Figure 3 shows the correlation between the affinity (log K_i) and agonist potency (percent of a 1 μM domoate response produced by 10 μM of the test compound) of the 4-(α-arylvinyl) kainates. The effect at a concentration of only 10 μM was chosen for comparison because some derivatives (for example, **25** and **28**) appeared to produce a reduced maximum response from that produced by other 4-(α-arylvinyl) kainates and domoate. Compounds which had low receptor affinity and no functional activity did not behave as antagonists (data not shown).

Several of the 4-(α-arylvinyl) kainates, including **25**, were evaluated for their ability to produce kainic acid-like clonic/tonic convulsions in mice by the intracerebral route of administration. All compounds tested produced

Table 2

	[^{3}H]kainate binding K_i (nM)		electrophysiology, humGluR6 receptors, % of domoate (1 μM) response		
compd	humGluR6 receptors	rat forebrain receptors	1 μM	10 μM	30 μM
11	29.8 ± 6.9	1.6 ± 0.25	48 ± 4	81 ± 10	80 ± 4
12	122.5 ± 40.9	2.2 ± 1.03	29 ± 7	75 ± 20	74 ± 18
13	41.1 ± 1.8	2.6 ± 0.87	61 ± 6	72 ± 13	76 ± 12
14	93.5 ± 21.5	3.5 ± 0.45	46 ±14	77 ± 7	87 ± 12
15	57.4 ± 15.4	3.9 ± 1.61	37 ±14	76 ± 15	82 ± 13
16	31.7 ± 5.4	3.9 ± 0.31	52 ± 2	78 ± 4	83 ± 4
17	38.4 ± 21.7	2.7 ± 0.49	43 ± 8	76 ± 4	78 ± 5
18	107.8 ± 20.4	2.3 ± 0.90	50 ± 4	89 ± 11	98 ± 14
19	169.3 ± 23.6	9.5 ± 1.43	23 ± 8	73 ± 5	108 ± 6
20	54.3 ± 16.2	1.9 ± 0.63	38 ± 9	76 ± 8	100 ± 15
21	82.2 ± 24.3	7.7 ± 3.02	22 ± 6	63 ± 7	73 ± 6
22	146.8 ± 46.4	3.6 ± 0.67	24 ± 11	54 ± 6	66 ± 7
23	145.6 ± 26.2	4.8 ± 2.80	11 ± 3	57 ± 9	63 ± 10
24	262.7 ± 59.7	18.5 ±12.75	8 ± 3	31 ± 5	46 ± 7
25	131.5 ± 46.2	3.6 ± 0.59	17 ± 3	61 ± 6	66 ± 10
26	288.0 ± 105.0	9.0 ± 0.83	15 ± 9	38 ± 5	52 ± 5
27	90.6 ± 10.9	2.1 ± 0.57	65 ± 3	98 ± 2	103 ± 5
28	130.6 ± 29.8	9.1 ± 3.18	36 ± 1	58 ± 1	66 ± 4
29	275.3 ± 598.5	23.3 ± 0.47	5 ± 7	8 ± 7	40 ± 6
30	146.6 ± 52.0	2.8 ± 0.64	33 ± 3	86 ± 5	99 ± 3
31	209.6 ± 60.9	11.9 ± 2.29	15 ± 0	59 ± 5	62 ± 2
32	667.0 ± 259.8	16.0 ± 6.52	6 ± 1	43 ±11	70 ± 6
33	126.2 ± 40.3	6.2 ± 1.10	16 ± 7	52 ± 5	66 ± 6
34	1295.7 ± 272.2	126.0 ± 34.9	0 ± 0	13 ± 12	27 ± 4
35	2423.3 ± 300.2	210.3 ± 59.3	3 ± 1	6 ± 1	14 ± 4
36	1284 ± 131.5		2 ± 2	20 ± 1	44 ± 6
37	129.3 ± 13.6		12 ± 4	64 ± 6	71 ± 5
38	663.3 ± 300.1		17 ± 7	46 ± 4	58 ± 6
39	6969.0 ± 1330.8	230.0 ± 38.3	*a*	*a*	*a*
40	2303.3 ± 802.5	5400 and 2400			
41	3109.2 ± 934.3		0 ± 0	0 ± 0	0 ± 0
42	>100000	3000 ± 100	0 ± 0	0 ± 0	0 ± 0
43	>100000		0 ± 0	0 ± 0	0 ± 2
44	>100000		12 ± 2	14 ± 3	16 ± 12
kainic acid	53.0 ± 23.6	3.7 ± 0.20	39 ± 5	85 ± 2	101 ± 7
domoate	18.3 ± 10.2				

[a] Compound **39** was neurotoxic to cells at these concentrations.

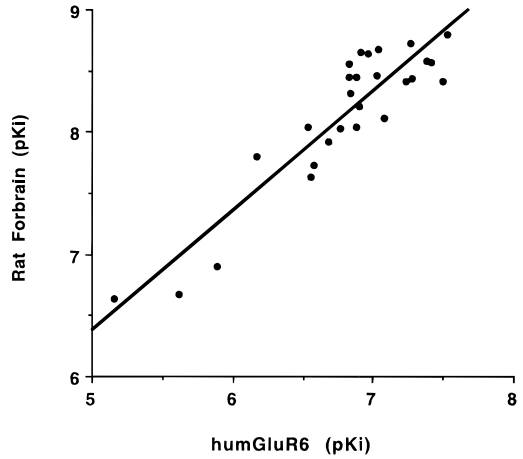


Figure 2. Correlation between the pK_i's for the binding of [^{3}H]kainic acid to humGluR6 and rat forebrain receptors (r^2 = 0.83, p < 0.001).

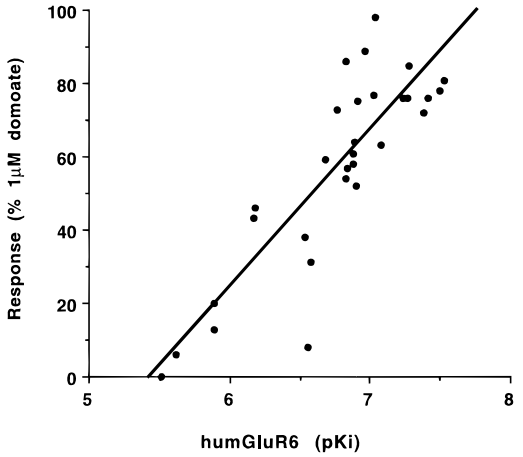


Figure 3. Correlation between the pK_i's for the binding of [^{3}H]kainic acid to humGluR6 and the electropysiological activity of 10 μM of the compounds expressed as a percentage of the response evoked by 1 μM domoate (r^2 = 0.73, p < 0.001).

such seizures. Compound **11** produced seizures in 100% of the mice at a dose of 1 nmol. These data further confirm the kainic acid-like nature of the 4-(arylvinyl) kainates and likely potential to produce neurodegeneration. All of the compounds identified in Table 2, with the exception of **40**, have been evaluated for affinity for the AMPA humGluR2 receptor. All have markedly lower affinity for this recombinantly expressed AMPA receptor than for the humGluR6 kainate receptor. The K_i of **11** for the humGluR2 AMPA receptor was 1263 ± 567 nM. Compound **11** was also evaluated for affinity for NMDA receptors (displacement of ^{3}[H]CGS 19755 binding[32]). Its K_i was greater than 50 μM.

In summary, the structure−activity relationships of a novel series of kainate receptor agonists have been thoroughly characterized for the first time in both recombinantly expressed and native kainate receptors. The failure to discover compounds with antagonist activity was disappointing; however, these studies uncovered a portion of the kainic acid molecule which could be significantly altered, including compounds with

substantially increased size and lipophilic character, without markedly affecting kainate receptor affinity or intrinsic activity.

Experimental Section

Chemistry. Melting points were obtained using a Thomas-Hoover capillary melting point apparatus and are uncorrected. ^{1}H NMR spectra were obtained at 300.15 MHz with TMS as an internal standard. Field absorption mass spectroscopy (FDMS) was performed using either a VG 70SE or Varian MAT 731 instrument. Analytical silica gel HPLC was performed on a Rainin HPLC instrument utilizing a 4.6 × 250 mm steel column (Microsorb, 5 m SiO_2, 100 Å pore size). Eluent systems are provided for the individual samples. Preparative HPLC was performed with the Waters Prep LC/2000 apparatus using dual silica gel prep-pack cartridges. Gradient solvent systems were employed as listed in the particular sample. Preparative centrifugal thin-layer chromatography (PC-TLC) was performed on a Harrison Model 7924A chromatotron using Analtech silica gel GF rotors. The plate thickness and solvent employed are indicated in the particular example. Column chromatography was performed by gravitational flow with use of Selecto Scientific silica gel (100−200 μm). Cation-exchange chromatography was performed using Dowex 50XB-100, 50−100 dry mesh resin. Eluent systems are provided for particular samples. Thin layer chromatography (TLC) was performed using silica gel-coated glass plates (EM Science, 5 × 20 cm, 0.25 mm layer thickness) and employing the solvent system indicated in the particular example. All temperatures are internal temperatures unless otherwise stated. For compounds **11**−**44** ^{1}H NMR and mass spectra were consistent with assigned structures, and elemental analysis (C, H, N) for all final products were within 0.4% of theoretical values. Intermediate compounds were characterized by ^{1}H NMR and mass spectra which were consistent with assigned structures.

(1*SR*,2*SR*,5*SR*)-Ethyl *N*-(Benzyloxycarbonyl)-3-aza-6-phenylbicyclo[3.3.0]oct-6-ene-2-carboxylate (45) and General Procedure for the Synthesis of Substituted Phenyl Derivatives 6. A solution of ethyl ether (220 mL) and methylene chloride (200 mL) at −78 °C under N_2 was treated sequentially with titanium tetrachloride (100 mL of a 1 M solution in methylene chloride, 0.1 mol) and phenyl magnesium bromide (34 mL of a 3.0 M solution in ethyl ether, 0.1 mol). The resulting dark solution was allowed to stir at −78 °C for 30 min. A solution of **5** (16.56 g, 0.05 mol in 200 mL of methylene chloride) was then added dropwise over a 50 min period. After the addition, the solution was allowed to warm slowly to 0 °C and remain there for 2 h, at which time TLC analysis, hexane/ethyl acetate (2:1), revealed complete consumption of **5**. The reaction mixture was poured into 300 mL of water and diluted with an additional 300 mL of ethyl ether. The layers were separated and the aqueous layer was washed with 300 mL of ethyl ether. The combined organic layers were washed once with water, dried over K_2CO_3, and concentrated *in vacuo* to a yellow oil (21.34 g). TLC analysis, hexane/ethyl acetate (2:1), revealed a 1:1 mixture of desired product and the intermediate carbinol. This was placed into 200 mL of toluene and 500 mg of *p*-toluenesulfonic acid, the solution was heated at reflux for 3.5 h, and the water was isolated via a Dean-Stark trap. The solution was cooled, washed twice with water, dried over K_2CO_3, and concentrated *in vacuo* producing a dark oil (20.02 g). Purification by preparative HPLC (gradient elution using hexane/ethyl acetate (19:1) to hexane/ethyl acetate (3:1)) gave **45** as a light oil (17.47 g, 89%): FDMS 391 $(M + H)^+$. Anal. ($C_{24}H_{25}NO_4$) C, H, N.

(2*SR*,3*SR*,4*SR*)-*N*-(Benzyloxycarbonyl)-2-(ethoxycarbonyl)-4-benzoylpyrrolidine-3-acetaldehyde (46) and General Procedure for the Synthesis of Substituted Phenyl Derivatives 7. A vigorously stirred solution of **45** (5.7 g, 15 mmol) in methylene chloride (250 mL) was subjected at −78 °C to generated O_3 dispersion until a dark blue color remained for 15 min. Nitrogen was then dispersed through the solution until the reaction mixture became colorless. Methyl sulfide (10.6 mL, 10 equiv) was then added, and the solution was allowed to warm to room temperature and stirred for 16 h. The reaction mixture was concentrated *in vacuo* producing a viscous yellow oil (5.82 g). Purification was achieved by flash silica gel chromatography using a gradient elution of hexane/ethyl acetate (19:1) to hexane/ethyl acetate (3:1) which gave **46** as a colorless oil (4.97 g, 79%): FDMS 423 $(M + H)^+$. Anal. ($C_{24}H_{25}NO_6 \cdot 0.5H_2O$) C, H, N.

(2*SR*,3*SR*,4*SR*)-Methyl *N*-(Benzyloxycarbonyl)-2-(ethoxycarbonyl)-4-benzoylpyrrolidine-3-acetate (48) and General Procedure for the Synthesis of Substituted Phenyl Derivatives 8. A solution of **46** (14.0 g, 36 mmol) in *tert*-butyl alcohol (222 mL) at room temperature was treated sequentially with 5% aqueous NaH_2PO_4 (148 mL) and 1.0 M $KMnO_4$ (222 mL). The resulting purple solution was stirred at room temperature for 1 h. A saturated aqueous solution of Na_2SO_3 (183 mL) was added dropwise, and the reaction mixture was partitioned between 1 L of 0.5 N HCl and 1 L of ethyl ether. The layers became colorless, and the aqueous layer was extracted with 500 mL of ethyl ether. The combined organic layers were washed once with water, dried over $MgSO_4$, and concentrated *in vacuo* producing the intermediate carboxcyclic acid **47** as a white foam (15.0 g, 95%): FDMS 439 $(M + H)^+$. Anal. ($C_{24}H_{25}NO_7$) C, H, N.

To this 15.0 g (34 mmol) and cesium carbonate (12.36 g, 38 mmol) in DMF (135 mL) at 0 °C was added dropwise methyl iodide (20.37 g, 170 mmol). The reaction mixture was allowed to warm to room temperature, stirred for 1.5 h, poured into 500 mL of 0.1 N HCl, and extracted three times with ethyl ether (200 mL). The ethyl ether layer was washed once with water, dried over K_2CO_3, and concentrated *in vacuo* to yield a viscous yellow oil (13.79 g). Purification by preparative HPLC (gradient elution of hexane/ethyl acetate (9:1) to hexane/ethyl acetate (1:1)) gave **48** as a colorless oil (11.09 g, 72%): FDMS 453 $(M + H)^+$. Anal. ($C_{25}H_{27}NO_7$) C, H, N.

(2*SR*,3*SR*,4*SR*)-*N*-(Benzyloxycarbonyl)-2-carboxy-4-(1-phenylethen-1-yl)pyrrolidine-3-acetic Acid (49), (2*SR*,3*SR*,4*RS*)-*N*-(Benzyloxycarbonyl)-2-carboxy-4-(1-phenylethen-1-yl)pyrrolidine-3-acetic Acid (50), and General Procedures for the Synthesis of Substituted Phenyl Derivatives 9 and 10. To a stirred suspension of methyltriphenylphosphonium bromide (24.38 g, 68 mmol) in toluene (320 mL) at 0 °C under nitrogen was added a 0.5 mmol (69 mL, 27 mmol) solution of potassium bis(trimethylsilyl)amide in toluene. Immediately, a solution of **47** (6.0 g, 13 mmol) in toluene (125 mL) was added dropwise (direct addition), and the reaction mixture was stirred an additional 2.5 h at 0 °C. TLC analysis, hexane/ethyl acetate (7:3), revealed the reaction to be complete. Water (200 mL) was added dropwise at 0 °C, and the mixture was further diluted with 200 mL of ethyl ether. The layers were separated, and the organic layer was washed once with water, dried over K_2CO_3, and concentrated *in vacuo* producing a brown oil (13.71 g). Purification by preparative HPLC, gradient elution of hexane/ethyl acetate (9:1) to hexane/ethyl acetate (7:3), gave 4.82 g (80%) of a yellow oil. Analytical silica gel HPLC, gradient elution of hexane/chloroform (19:1) to hexane/chloroform (1:1) (over 20 min), revealed it to be a mixture of the kainoid (**51**) and allo (**52**) esters in a 91:9 ratio, respectively, with the kainoid isomer eluting first.

The ratio of the allo ester **52** could be increased by the addition to a stirred suspension of methyltriphenylphosphonium bromide (20.30 g, 55 mmol) in toluene (250 mL) at 0 °C under nitrogen a 0.5 M solution of potassium bis(trimethylsilyl)amide in toluene (58 mL, 22 mmol). The reaction was allowed to continue at 0 °C for 30 min. This mixture was added (via Teflon cannula) dropwise to a stirred solution of **47** until the reaction was complete using TLC, hexane/ethyl acetate (7:3). Water (200 mL) was added dropwise at 0 °C followed by ethyl ether. The layers were separated, and the organic layer was washed once with water, dried over K_2CO_3, and concentrated *in vacuo* producing a brown oil (7.49 g). Purification by preparative HPLC (elution with hexane/ethyl acetate (9:1) to hexane/ethyl acetate (7:3)) gave 3.37 g of a yellow oil. Analytical silica gel HPLC, gradient elution of hexane/chloroform (19:1) to hexane/chloroform (1:1) over 20 min, revealed it to be a mixture of the kainoid (**51**) and allo

(**52**) esters in a 36:64 ratio, respectively, with the kainoid isomer eluting first: FDMS 451 $(M + H)^+$. Anal. ($C_{26}H_{29}NO_6$) C, H, N.

The mixtures of **51** and **52** (4.60 g, 10 mmol) were stirred in 1 N sodium hydroxide (60 mL) and tetrahydrofuran (60 mL) for 3 days at room temperature until TLC analysis, ethyl/acetate methanol (1:1), showed the reaction to be complete. The mixtures were concentrated *in vacuo*, and the pH was adjusted to 3.0 using 1 N hydrochloric acid. The desired product were extracted with ethyl acetate, and the organic layer was washed one time with water, dried over $MgSO_4$, and concentrated *in vacuo* producing a white foam (4.17 g). Analytical silica gel HPLC, gradient elution of hexane/acetic acid (97:3) to chloroform/acetic acid (97:3) over 30 min, revealed the ratio of kainoid and allo isomers to be unchanged. Separation and purification of the isomers were achieved by preparative HPLC, gradient elution of hexane/chloroform/acetic acid (87:10:3) to hexane/chloroform/acetic acid (10:87:3), producing pure kainoid isomer **49** (which eluted first) and pure allo isomer **50** as foams. For compound **49**: FDMS 409 $(M + H)^+$. For compound **50**: FDMS 409 $(M + H)^+$. Anal. ($C_{23}H_{23}NO_6 \cdot 0.5H_2O$) C, H, N.

(2*SR*,3*SR*,4*SR*)-2-Carboxy-4-(1-phenylethen-1-yl)pyrrolidine-3-acetic Acid (11) and General Procedure for the Synthesis of Substituted Phenyl Derivatives 1. A mixture of **49** (1.50 g, 3.7 mmol), 50% sodium hydroxide (25 mL), and methanol (25 mL) was refluxed for 16 h with vigorous stirring until TLC analysis, chloroform/methanol/water/ammonium hydroxide (45:45:9:1), showed the reaction to be complete. The reaction mixture was cooled to room temperature and concentrated *in vacuo*. The resulting residue was diluted with 25 mL of water, and the pH was adjusted to 1.0 using concentrated hydrochloric acid and ice. This mixture was washed one time with ethyl acetate, and the pH was adjusted to 7.0 with 1 N sodium hydroxide. The aqueous media was concentrated *in vacuo* producing a white solid containing desired product and inorganic salts. This was extracted three times with methanol (300 mL), and the extracts were concentrated *in vacuo* to produce a white solid (6.01 g). This material was subjected to cation-exchange chromatography (Dowex 50XB-100, 50−100 dry mesh resin) utilizing 10% aqueous pyridine as the eluent. The resulting solid was recrystallized from water producing **11** (410 mg, 42%): mp 162.5−164.5 °C; FDMS 276 $(M + H)^+$. Anal. ($C_{15}H_{17}NO_4$) C, H, N.

(2*SR*,3*SR*,4*RS*)-2-Carboxy-4-(1-phenylethen-1-yl)pyrrolidine-3-acetic Acid (39) and General Procedure for the Synthesis of Substituted Phenyl Derivatives 2. A mixture of **50** (1.0 g, 2.5 mmol), 50% sodium hydroxide (25 mL), and methanol (25 mL) was reacted following the procedure used to prepare **11**. Cation-exchange chromatography and recrystallization from water produced **39** (433 mg, 65%): mp dec>195 °C; FDMS 276 $(M + H)^+$. Anal. ($C_{15}H_{17}NO_4$) C, H, N.

(2*SR*,3*SR*,4*SR*)-Carboxy-4-(1-(*SR*)- and 1-(*RS*)-phenyleth-1-yl)pyrrolidine-3-acetic Acid (43). A mixture of **49** (1.50 g, 3.7 mmol) and 5% Pd/C (0.375 g) in ethanol (50 mL) was subjected to hydrogenation at room temperature overnight at a pressure of 60 psi. The solution was filtered, the resulting material was placed into 100 mL of water, and the pH was adjusted to 2.0 using 1 N hydrochloric acid. A white precipitate formed and was collected by filtration (600 mg). Recrystallization from water/acetonitrile (3:1) yielded **43** (530 mg, 41%): mp >260 °C dec; FDMS 277 $(M + H)^+$. Anal. ($C_{15}H_{19}NO_4$) C, H, N.

(2*SR*,3*SR*,4*SR*)-2-Carboxy-4-[1-(*SR*)- and 1-(*RS*)-[3-(3-phenylphenyl]eth-1-yl]pyrrolidine-3-acetic Acid (44). The procedure described for the synthesis of **43** was used. Recrystallization from water/acetonitrile (1:5) yielded **44**: mp >166 °C dec; FDMS 354 $(M + H)^+$. Anal. ($C_{21}H_{23}NO_4$) C, H, N.

Biology: humGluR6-Transfected HEK293 Cells. For stable expression of humGluR6 in HEK293 cells, cDNA coding for humGluR6 receptor was incorporated into the mammalian expression vector pRc/CMV (invitrogen). HEK293 cells were transfected with 3 μg DNA (as pRC/CMV-hum) per 4×10^5 cells, by lipofectin (GIBCO-BRL)-mediated gene transfer. Cells resistant to neomycin were selected in 10% FBS-supplemented α-MEM medium containing G418. Individual colonies of G418 resistant cells were isolated about 2−3 weeks later. The cell line designated EAA4-9 was established and propogated for use.[14] Cells were dissociated by trituration and then plated onto glass coverslips.

[^{3}H]Kainate Binding. Binding studies to rat forebrain receptors were performed essentially as previously described.[33] For binding to the humGluR6 kainate receptor[14] frozen EAA4-9 cells were lysed, by suspension in ice-cold purified water, and then centrifuged for 20 min at 50000*g*. The resulting membrane pellets were used the same day or stored frozen at −80 °C for 24 h. For binding assays, in order to remove endogenous glutamate, the membrane preparations were washed by resuspending in >100 vol of 50 mM Tris-HCl buffer, pH 7.5 at 5 °C, and centrifuged for 10 min at 50000*g*.

[^{3}H]Kainate binding experiments were performed by incubating washed membranes (100−150 μg of protein/sample) with [^{3}H]kainate (30 nM) in the same buffer as used for washing in a total volume of 0.25 mL. L-Glutamate (1 mM) was used to define nonspecific binding. The binding reaction was performed by incubation on ice for 60 min after the addition of the membrane suspension. Bound ligand was separated from free ligand by centrifugation. The saturation and Scatchard data were analyzed with the aid of the computer software program GRAFIT.

Electrophysiological Recordings. Whole cell voltage clamp recordings were made from HEK293 cells transfected with humGluR6 using the tight seal whole cell configuration of the patch-clamp technique.[32] Glass fragments of coverslips with adherent cells were placed in a perfusion recording chamber and rinsed thoroughly with recording buffer of the following composition (in mM): NaCl, 138; $CaCl_2$, 5; $MgCl_2$, 1; KCl, 5; HEPES, 10; glucose, 10; adjusted to pH 7.4 with NaOH. Pipet solutions contained (in mM) KCl, 140; $MgCl_2$, 1; HEPES, 10; EGTA, 0.1; pH 7.2 with KOH. Currents were recorded on either an Axopatch ID or List EPC-7 amplifier and displayed on a chart recorder. Pipet resistances of 1.5−2.5 MΩ were used, and currents were filtered through an 8-pole Bessel filter.

Drug application was via bath perfusion, and exchange of solutions occurred in approximately 20 s. Experiments using domoate in HEK293 cells expressing humGluR6 were performed in the presence of 2.5 μM conconavalin A to prevent agonist-induced humGluR6 receptor desensitization. Results are expressed as the percent of a 1 μM domoate[17,34] response in the presence of 2.5 μM concanavalin A.

Supporting Information Available: Crystal data for **11** and tables listing atomic coordinates, bond lengths, bond angles, anisotropic displacement coefficients, H-atom coordinates, isotropic displacement coefficients, data collection methods, solution, and refinement (8 pages). Ordering information is found on any current masthead page.

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