Ethyl (3*S*,4a*R*,6*S*,8a*R*)-6-(4-Ethoxycarbonylimidazol-1-ylmethyl)decahydroisoquinoline-3-carboxylic Ester: A Prodrug of a GluR5 Kainate Receptor Antagonist Active in Two Animal Models of Acute Migraine

Sandra A. Filla,*[,†] Mark A. Winter,[†] Kirk W. Johnson,[†] David Bleakman,[†] Michael G. Bell,[†] Thomas J. Bleisch,[†] Ana M. Castaño,[‡] Amy Clemens-Smith,[†] Miriam del Prado,[‡] Donna K. Dieckman,[†] Esteban Dominguez,[‡] Ana Escribano,[‡] Ken H. Ho,[§,||] Kevin J. Hudziak,[†] Mary A. Katofiasc,[†] Jose A. Martinez-Perez,[‡] Ana Mateo,[‡] Brian M. Mathes,[†] Edward L. Mattiuz,[†] Ann Marie L. Ogden,[†] Lee A. Phebus,[†] Douglas R. Stack,[†] Robert E. Stratford,[†] and Paul L. Ornstein[†]

Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana, 46285, Centro de Investigación Lilly, S. A. Avda. De la Industria 30, 28108-Alcobendas, Madrid, Spain, and Allelix Biopharmaceuticals, Mississauga, Ontario L4V1P1, Canada

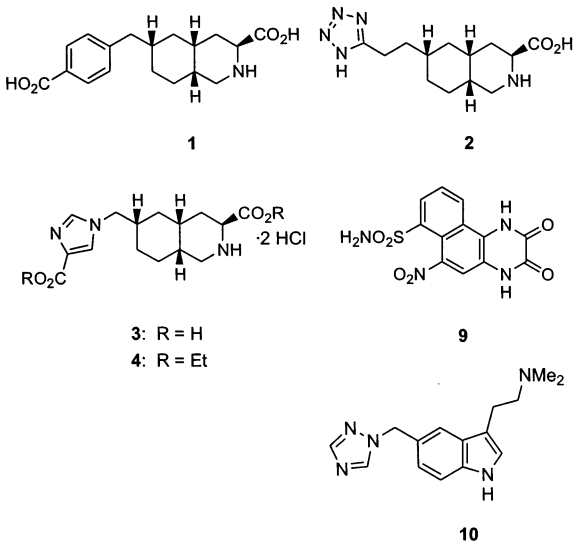
Received June 4, 2002

Abstract: Amino diacid **3**, a highly selective competitive GluR5 kainate receptor antagonist, exhibited high GluR5 receptor affinity and selectivity over other glutamate receptors. Its diethyl ester prodrug **4** was orally active in two models of migraine: the neurogenic dural plasma protein extravasation model and the nucleus caudalis c-fos expression model. These data suggest that a GluR5 kainate receptor antagonist might be an efficacious antimigraine therapy with a novel mechanism of action.

Glutamic acid is the primary excitatory amino acid in the central nervous system. It mediates neuronal effects through two types of receptors: G-protein-coupled metabotropic glutamate receptors and ionotropic glutamate receptors, which are ligand-gated ion channels. The ionotropic receptors are defined, on the basis of subtype selective agonists, as *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), or kainate (KA).[1] Recent molecular biology studies have further divided the AMPA subtype into GluR1−4, and the KA subtype into GluR5−7 and KA1 and KA2 subunits.[2]

Several studies have implicated KA receptors, specifically the GluR5 receptor subtype, in pain transmission.[3] C-fiber primary afferents, which possess KA receptors,[4] transfer nociceptive information from the periphery to the spinal cord. The biophysical and pharmacological profile of KA receptors in the dorsal root ganglion (DRG), cell bodies in the dorsal root associated with primary afferent neurons, suggests that they are GluR5 receptors.[5] Simmons et al. demonstrated that the selective GluR5 KA receptor antagonist **1** (LY382884)[6] was active in an animal model of persistent pain,[7] and Sang and co-workers showed that **2** (LY293558),[8] a competitive AMPA/KA antagonist, significantly reduced pain intensity and unpleasantness in an experimentally induced human pain study and reduced clinical pain in a study of evoked pain (Chart 1).[9]

Chart 1



More recently, glutamate receptors have been linked with migraine headache. Investigators identified what appeared to be GluR5 KA receptors in the trigeminal ganglion neurons,[10] where pseudounipolar neurons innervating the meninges originate. Additionally, plasma levels of glutamate were elevated in patients with a history of migraine headaches, and glutamate levels were further raised in these patients during a migraine attack.[11] Recently, glutamate receptors were reported to modulate capsaicin-induced c-fos expression in the trigeminal nucleus caudalis,[12] an established model of migraine.[13] These data suggested that NMDA and AMPA, but not KA, receptor antagonists and mGluR receptor agonists might be effective acute treatments for migraine pain via a nonserotonergic mechanism of action.[14] In contrast, we propose that GluR5 KA receptor antagonists might be potential antimigraine agents. We describe here the in vitro activity of (3*S*,4a*R*,6*S*,8a*R*)-6-(4-carboxyimidazol-1-ylmethyl)decahydroisoquinoline-3-carboxylic acid **3**, a potent and selective GluR5 KA receptor antagonist, and the oral activity of diethyl ester **4**, in two animal models of migraine.

The preparation of compounds **3** and **4** is outlined in Scheme 1. Both compounds were derived from hydroxymethyl intermediate **5**, the synthesis of which has previously been described.[15] Conversion to nosylate **6**, followed by alkylation with ethyl imidazole-4-carboxylate, provided a 1:1 mixture of isomers **7** and **8**, which were separated by column chromatography. Treatment of the desired regioisomer **8** with TMSI selectively removed the methyl carbamate protecting group.

The resulting amine was then converted to the diethyl ester dihydrochloride salt **4**. Additionally, ester

* To whom correspondence should be addressed. Phone: 317-277-2789. Fax: 317-276-7600. E-mail: filla_sandra_a@lilly.com.
† Lilly Research Laboratories.
‡ Centro de Investigación Lilly.
§ Allelix Biopharmaceuticals.
|| Present address: Lilly Research Laboratories.

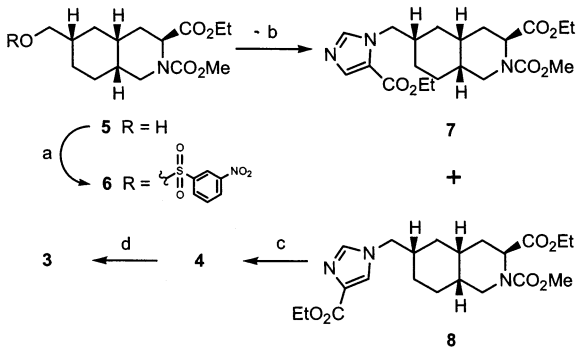
10.1021/jm025548q CCC: $22.00
Published on Web 08/24/2002

Table 1. In Vitro Binding Affinities of AMPA/KA Antagonists for Recombinant Human AMPA and KA Receptors[a]

compd	GluR1[b]	GluR2[b]	GluR3[b]	GluR4[b]	GluR5[c]	GluR6[c]	GluR7[c]	GluR6/KA2[c]
2	9.2 ± 3.9	3.2 ± 0.3	32 ± 5	50 ± 13	4.2 ± 0.3	>100	>100	>100
3	134 ± 19	117 ± 16	247 ± 33	262 ± 61	0.156 ± 0.075	>1000	48.5 ± 1.9	236 ± 27
9	1.6 ± 0.9	0.26 ± 0.04	0.9 ± 0.3	0.6 ± 0.9	11.6 ± 4.4	13.2 ± 1.9	23.9 ± 6.9	>100

[a] Affinities for receptors (K_i, μM) were determined in vitro by radioligand binding assays using HEK 293 cell membranes expressing the appropriate human AMPA or KA receptor.[16] Each value is the mean ± SEM of at least three determinations. [b] [^{3}H]AMPA was used as the high-affinity radioligand. [c] [^{3}H]KA was used as the high-affinity radioligand.

Scheme 1[a]



[a] Conditions: (a) NsCl, Et_3N, CH_2Cl_2, room temp (68%); (b) ethyl imidazole-4-carboxylate, NaH, DMF, room temp (40%); (c) (i) TMSI, CH_2Cl_2, room temp; (ii) HCl/Et_2O, room temp (72%, two steps); (d) 5 N HCl, 75 °C (85%).

deprotection of **4** by heating in 5 N HCl provided amino diacid **3**.

Amino diacid **3** was evaluated in ligand binding studies for its ability to displace binding of [^{3}H]AMPA to recombinant human AMPA receptors and [^{3}H]KA to recombinant human KA receptors expressed in HEK 293 cell membranes.[16] Table 1 shows the affinity of **3** for the various cloned human AMPA and KA receptors compared with competitive AMPA/KA antagonist **2** and the quinoxalidinedione AMPA/KA antagonist **9** (NBQX), a standard tool used in the study of AMPA receptors.[17] Compound **2** displaced binding to AMPA and GluR5 receptors but not other KA receptor subunits. As reported, **9** displaced radioligand binding at AMPA and to a lesser extent KA receptors and showed no selectivity for the GluR5 receptor. In contrast, **3** exhibited high affinity for the KA GluR5 receptor, with greater than 300-fold selectivity over other KA and AMPA receptors.

Compound **3** behaved as an antagonist, inhibiting glutamate-evoked calcium influx in the human GluR5-(Q) receptor stably transfected in HEK293 cell membranes, with an estimated IC_{50} of 1.18 ± 0.22 μM (Figure 1).[16,18] Consistent with competitive antagonists in this assay, **3** produced rightward shifts in the glutamate concentration−effect curves, with an estimated K_B of 0.259 ± 0.063 μM (Figure 2).[19] In addition, the inhibitory activity of **3** was evaluated at GluR5 KA receptors found in rat neonatal DRG neurons, using whole cell voltage clamp recordings.[20] Its IC_{50} value, determined against a screening concentration of 30 μM KA, was estimated to be 0.85 ± 0.11 μM. On the basis of these data, we characterized **3** as a highly selective, competitive GluR5 KA receptor antagonist.

We evaluated the oral efficacy of this novel GluR5 KA receptor antagonist in two animal models of migraine. Parent amino diacid **3** itself exhibited poor oral bioavailability in rat; consequently we evaluated diester **4** as a potential prodrug of **3**. We did not detect **4** in rat

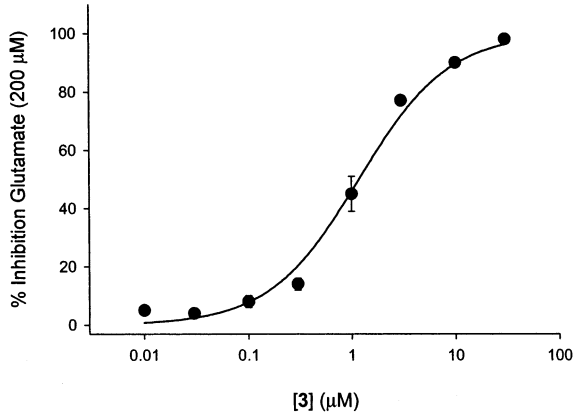


Figure 1. Inhibition of glutamate (200 μM)-invoked calcium influx by **3** in the human GluR5(Q) receptor stably transfected in HEK293 cells, with mean value ± SEM for $n = 3$.

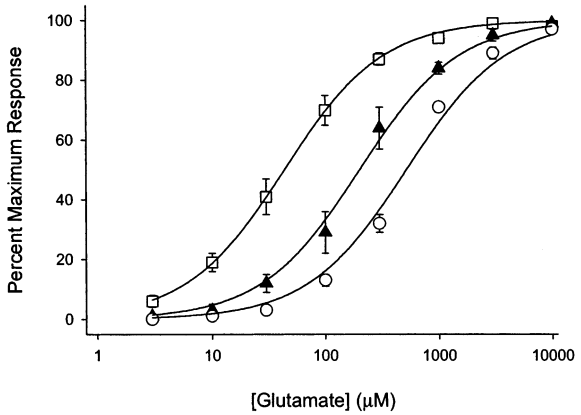


Figure 2. Effects of **3** on the concentration−effect curve of glutamate in the human GluR5(Q) receptor stably transfected in HEK293 cells: (□) glutamate (estimated EC_{50} of 54.96 ± 8.28 μM); (▲) glutamate + 300 nM **3**; (○) glutamate + 3 μM **3**. Included is the mean value ± SEM for $n = 3$.

plasma 1 h following a 30 mg/kg oral dose.[21] The plasma half-life of **3** following oral administration of **4** ranged from 2 to 3 h, and its oral biovailability, administered as its diethyl ester **4**, was determined to be 50%. On the basis of these results, we concluded that diethyl ester **4** would act as a prodrug of amino diacid **3**.

The neurogenic dural plasma protein extravasation (PPE) model, which examines the inhibition of neurotransmitter release from the peripheral branches of trigeminal sensory neurons following activation of the trigeminal nerve, is a widely accepted model of acute migraine.[22] Compounds with demonstrated clinical efficacy, such as sumatriptan, zolmitriptan, ergotamine, and rizatriptan (**10**), were effective at blocking plasma extravasation in this model.[23] The efficacy of **4** in the PPE model was compared to that of 5-HT_1 receptor agonist **10** following oral administration.[24] Compound **4**, administered orally to fasted Wistar rats 1 h prior to trigeminal stimulation, decreased extravasation in this

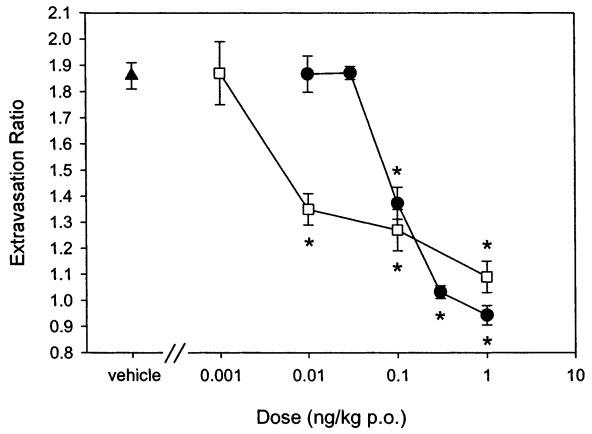


Figure 3. Dose−response curves of **4** and **10** in the PPE model after oral administration: (▲) vehicle; (●) **4**; (□) **10**. Values are the mean ratio of Evan's Blue dye extravasation in the stimulated side of the dura to that in the unstimulated side (±SEM) for $n = 3-6$. Asterisks denote a significant difference from the vehicle group ($P < 0.05$).

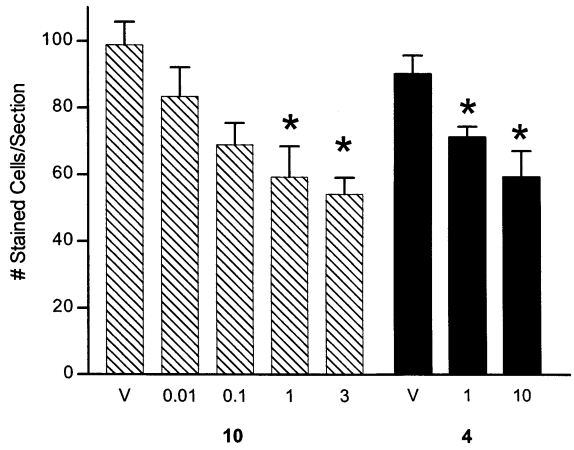


Figure 4. Comparison of **4** and **10** in the suppression of c-fos expression in the rat nucleus caudalis ipsilateral to trigeminal ganglion stimulation (V = vehicle). For each animal, 10−15 sections were analyzed to provide an average number of stained cells per section. These averages were combined for vehicle and compound pretreated animals ($n = 5-22$ animals/group). Asterisks denote a significant difference from the respective vehicle group ($P < 0.05$).

model in a dose-related manner, with an estimated ID_{50} of 100 pg/kg (determined 15 min after stimulation). Compound **10**, evaluated in the identical manner as **4**, had an estimated ID_{50} of 8 pg/kg (Figure 3). In addition, parent amino diacid **3** itself was evaluated 15 min following intravenous administration. Compound **3**, administered intravenously 10 min prior to trigeminal stimulation, decreased extravasation in a dose-related manner with an estimated ID_{50} of 0.03 pg/kg (data not shown). These data further supported our hypothesis that **4** was acting as a prodrug of **3**.

We also evaluated diester **4** in the nucleus caudalus c-fos expression model, which examines the inhibition of central neurotransmitter release and subsequent nociceptive signaling to higher brain centers, where pain is perceived. Unilateral electrical stimulation of the trigeminal sensory neurons induces ipsilateral Fos protein expression that has been used as a marker of neuronal activity and subsequently as an index of migraine-like pain transmission to the central nervous system.[18,25] As shown in Figure 4, **4** was comparable to **10** in decreasing the number of brainstem nucleus caudalis cells staining for Fos protein expression ipsilateral to the stimulation. One hour of oral pretreatment with 10 mg/kg dose of **4** produced an approximate 30% decrease in central Fos expression, compared to a 1 or 3 mg/kg dose of **10** to produce a similar effect.

Both **4** and **10** were considerably more potent in the PPE model than in the c-fos expression assay. This difference is often observed between the two models, regardless of the pharmacological mechanism of action of the test compound. The PPE model most likely examines the inhibition of neurotransmitter release in the periphery, whereas the c-fos assay is assessing inhibition of central neurotransmitter release. Thus, the degree of central penetration of a compound could cause a difference in potency between the two models. Despite the extreme potency of compounds in the PPE model, an excellent correlation existed between potency in the PPE model and GluR5 KA antagonist activity (data not shown).

In summary, **4**, the diethyl ester prodrug of amino diacid **3,** demonstrated oral efficacy in two well-established models of migraine. On the basis of these data, we propose that **3**, a selective, competitive GluR5 KA receptor antagonist, may be clinically effective in treating acute migraine pain via a nonserotonergic mechanism of action, representing a significant breakthrough in the search for new, safe therapies for migraine.

Supporting Information Available: Experimental procedures, including analytical and spectral data, for the preparation of compounds **3**, **4**, **6**, and **8**, as well as experimental details for the functional studies in recombinant human GluR5 KA receptors and for the nucleus caudalis c-fos expression model. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Collingridge, G. J.; Lester, A. J. Excitatory amino acid receptors in the vertebrate central nervous system. *Pharmacol. Rev.* **1989**, *40*, 145−195.

(2) (a) Bleakman, D.; Lodge, D. Neuropharmacology of AMPA and kainate receptors. *Neuropharmacology* **1998**, *37*, 1187−1204. (b) Hollman, M.; Heinemann, S. Cloned glutamate receptors. *Annu. Rev. Neurosci.* **1994**, *17*, 31−108. (c) Seeburg, P. H. The molecular biology of mammalian glutamate receptor channels. *Trends Neurosci.* **1993**, *16*, 359−365.

(3) (a) Li, P.; Wilding, T. J.; Kim, S. J.; Calejesan, A. A.; Huettner, J. E.; Zhuo, M. Kainate-receptor-mediated sensory synaptic transmission in mammalian spinal cord. *Nature* **1999**, *397*, 161−164. (b) Procter, M. J.; Houghton, A. K.; Faber, E. S. L.; Chizh, B. A.; Ornstein, P. L.; Lodge, D.; Headley, P. M. Actions of kainate and AMPA selective glutamate receptor ligands on nociceptive processing in the spinal cord. *Neuropharmacology* **1998**, *37*, 1287−1297. (c) Evans, R. H.; Long, S. K. Primary afferent depolarization in the rat spinal cord is mediated by pathways utilizing NMDA and non-NMDA receptors. *Neurosci. Lett.* **1989**, *100*, 231−236. (d) Evans R. H.; Evans, S. J.; Pook, P. C.; Sunter, D. C. A comparison of excitatory amino acid antagonists acting at primary afferent C fibers and motoneurons of the isolated spinal cord of the rat. *Br. J. Pharmacol.* **1987**, *91*, 531−537.

(4) Agrawal, S. G.; Evans, R. H. The primary afferent depolarizing action of kainate in the rat. *Br. J. Phamacol.* **1986**, *87*, 345−355.

(5) (a) Huettner, J. E. Glutamate receptor channels in DRG neurons: activation by kainate and quisqualate and blockade of desensitization by concanavalin. *Neuron* **1990**, *5*, 255−266. (b) Partin, K. M.; Patneau, D. K.; Winters, C. A.; Mayer, M. L.; Buonanno, A. Selective modulation of desensitization at AMPA versus kainate receptors by cyclothiazide and concanavalin A. *Neuron* **1993**, *11*, 1069−1082.

(6) Bleisch, T. J.; Ornstein, P. L.; Allen, N. K.; Wright, R. A.; Lodge, D.; Schoepp, D. D. Structure−Activity Studies of Aryl-Spaced Decahydroisoquinoline-3-carboxylic Acid AMPA Receptor Antagonists. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1161−1166.
(7) Simmons, R. M. A.; Li, D. L.; Hoo, K. H.; Deverill, M.; Ornstein, P. L.; Iyengar, S. Kainate GluR5 receptor subtype mediates the nociceptive response to formalin in the rat. *Neuropharmacology* **1998**, *37*, 25−36.
(8) (a) Ornstein, P. L.; Arnold, M. B.; Augenstein, N. K.; Lodge, D.; Leander, J. D.; Schoepp, D. D. (3*SR*,4a*RS*,6*RS*,8a*RS*)-6-[2(1*H*-Tetrazol-5-yl)ethyl]decahydroisoquinoline-3-carboxylic Acid: A Structurally Novel, Systemically Active, Competitive AMPA Receptor Antagonist. *J. Med. Chem.* **1993**, *36*, 2046−2048. (b) Ornstein, P. L.; Arnold, M. B.; Allen, N. K.; Bleisch, T.; Borromeo, P. S.; Lugar, C. W.; Leander, J. D.; Lodge, D.; Schoepp, D. D. Structure−Activity Studies of 6-(Tetrazolylalkyl)-Substituted Decahydroisoquinoline-3-carboxylic Acid AMPA Receptor Antagonists. 1. Effects of Stereochemistry, Chain Length, and Chain Substitution. *J. Med. Chem.* **1996**, *39*, 2219−2231. (c) Ornstein, P. L.; Arnold, M. B.; Allen, N. K.; Bleisch, T.; Borromeo, P. S.; Lugar, C. W.; Leander, J. D.; Lodge, D.; Schoepp, D. D. Structure−Activity Studies of 6-Substituted Decahydroisoquinoline-3-carboxylic Acid AMPA Receptor Antagonists. 2. Effects of Distal Acid Bioisosteric Substitution, Absolute Stereochemical Preferences, and in Vivo Activity. *J. Med. Chem.* **1996**, *39*, 2232−2244.
(9) (a) Sang, C. N.; Hostetter, M. P.; Gracely, R. H.; Chappell, A. S.; Schoepp, D. D.; Lee, G.; Whitcup, S.; Caruso, R.; Max, M. B. AMPA/Kainate Antagonist LY293558 Reduces Capsaicin-Evoked Hyperalgesia but Not Pain in Normal Skin in Humans. *Anesthesiology* **1998**, *89*, 1060−1067. (b) Gilron, I.; Max, M. B.; Lee, G.; Booher, S. L.; Sang, C. N.; Chappell, A. S.; Dionne, R. A. Effects of the 2-amino-3-hydroxy-5-methyl-isoxazole-proprionic acid/kainate antagonist LY293558 on spontaneous and evoked postoperative pain. *Clin. Pharmacol. Ther.* **2000**, *68*, 320−327.
(10) Sahara, Y.; Noro, N.; Iida, Y.; Soma, K.; Nakamura, Y. Glutamate Receptor Subunits GluR5 and KA-2 Are Coexpressed in Rat Trigeminal Ganglion Neurons. *J. Neurosci.* **1997**, *17*, 6611−6620.
(11) Alam, Z.; Coombes, N.; Waring, R. H.; Williams, A. C.; Steventon, G. B. Plasma levels of neuroexcitatory amino acids in patients with migraine or tension headache. *J. Neurol. Sci.* **1998**, *156*, 102−106.
(12) Mitsikostas, D. D.; Sanchez del Rio, M.; Waeber, C.; Huang, Z.; Cutrer, F. M.; Moskowitz, M. A. Non-NMDA glutamate receptors modulate capsaicin induced c-*fos* expression within trigeminal nucleus caudalis. *Br. J. Pharmacol.* **1999**, *127*, 623−630.
(13) Moskowitz, M. A.; MacFarlane, R. Neurovascular and molecular mechanisms in migraine headaches. *Cereb. Brain Metab. Rev.* **1993**, *5*, 159−177.
(14) For reviews on the role of serotonin in migraine, see: (a) Johnson, K. W.; Phebus, L. A.; Cohen, M. L. Serotonin in migraine: Theories, animal models and emerging therapies. In *Progress in Drug Research*; Jucker, E., Ed.; Birkhäuser Verlag: Basel, Switzerland, 1998; Vol. 51, pp 219−244. (b) Goadsby, P. J. Serotonin 5-HT1B/1D receptor agonists in migraine: comparative pharmacology and its therapeutic implications. *CNS Drugs* **1998**, *10*, 271−286. (c) DeVries, P.; Villalon, C. M.; Saxena, P. R. Pharmacology of triptans. *Emerging Drugs* **1999**, *4*, 107−125.
(15) Ornstein, P. L.; Augenstein, N. K.; Arnold, M. B. Stereoselective Synthesis of 6-Substituted Decahydroisoquinoline-3-carboxylates: Intermediates for the Preparation of Conformationally Constrained Acidic Amino Acids. *J. Org. Chem.* **1994**, *59*, 7862−7869.
(16) Small, B.; Thomas, J.; Kemp, M.; Hoo, K.; Ballyk, B.; Deverill, M.; Ogden, A. M.; Rubio, A.; Pedregal, C.; Bleakman, D. LY339434, a GluR5 kainate receptor agonist. *Neuropharmacology* **1998**, *37*, 1261−1267.
(17) Sheardown, M. J.; Nielsen, E. Ø.; Hansen, A. J.; Jacobsen, P.; Honoré, T. 2,3-Dihydroxy-6-nitro-7-sulfamoyl-benzo(F)quinoxaline: a neuroprotectant for cerebral ischemia. *Science* **1990**, *247*, 571−574.
(18) For experimental methods, see Supporting Information.
(19) The K_B for these experiments was estimated by nonlinear regression analysis of data represented in Figure 2. This method allows simultaneous fitting of agonist curves using the methods described in the following references. (a) Bemis, K. Fitting nonlinear models in isolated tissue studies using PROCNLIN in SAS. *American Statistical Association, Proceedings of the Biopharmaceutical Section* **1985**, 72−74. (b) Lew, M. J.; Angus, J. A. Analysis of competitive agonist-antagonist interactions by nonlinear regression. *Trends Pharmacol. Sci.* **1985**, *16*, 328−337.
(20) Bleakman, D.; Ogden, A.; Ornstein, P. L.; Hoo, K. Pharmacological characterization of a GluR6 kainate receptor in cultured hippocampal neurons. *J. Pharmacol.* **1999**, *378*, 331−337. (b) Bleakman, D.; Schoepp, D. D.; Ballyk, B.; Bufton, H.; Sharpe, E. F.; Thomas, K.; Ornstein, P. L.; Kamboj, R. K. Pharmacological Discrimination of GluR5 and GluR6 Kainate Receptor Subtypes by (3*S*,4a*R*,6*R*,8a*R*)-6-[2-(1(2)*H*-tetrazole-5-yl)ethyl]-decahydroisoquinoline-3-carboxylic-acid. *Mol. Pharmacol.* **1996**, *49*, 581−585.
(21) The in vitro binding affinity of diester **4** at all AMPA and KA receptors tested, including the GluR5 KA receptor, was greater than 40 μM.
(22) (a) Buzzi, M. G.; Bonamini, M.; Moskowitz, M. A. Neurogenic model of migraine. *Cephalalgia* **1995**, *15*, 277−280. (b) Moskowitz, M. A.; Cutrer, F. M.; Lee, W. S.; Waeber, C.; Limmroth, V.; Yu, X. Drug Discovery in Migraine Circa 1995. In *Experimental Headache Models*; Olesen, J., Moskowitz, M. A., Eds.; Lippincott-Raven Publishers: Philadelphia, PA, 1995; pp 63−73.
(23) (a) Buzzi, M. G.; Moskowitz, M. A. The antimigraine drug sumatriptan (GR43175), selectively blocks neurogenic plasma extravasation from blood vessels in dura matter. *Br. J. Pharmacol.* **1990**, *99*, 202−206. (b) Johnson, K. W.; Schaus, J. M.; Durkin, M. M.; Audia, J. E.; Kaldor, S. W.; Flaugh, M. E.; Adham, N.; Zgombick, J. M.; Cohen, M. L.; Branchek, T. A.; Phebus, L. A. 5-HT_{1F} receptor agonists inhibit neurogenic dural inflammation in guinea pigs. *NeuroReport* **1997**, *8*, 2237−2240.
(24) Street, L. J.; Baker, R.; Davey, W. B.; Guiblin, A. R.; Jelley, R. A.; Reeve, A. J.; Routledge, H.; Sternfeld, F.; Watt, A. P.; Beer, M. S.; Middlemiss, D. N.; Noble, A. J.; Stanton, J. A.; Scholey, K.; Hargreaves, R. J.; Sohal, B.; Graham, M. I.; Matassa, V. G. Synthesis and Serotonergic Activity of *N*,*N*-Dimethyl-2-[5-(1,2,4-triazol-1-ylmethyl)-1*H*-indol-3-yl]ethylamine and Analogues: Potent Agonists for 5-HT_{1D} Receptors. *J. Med. Chem.* **1995**, *38*, 1799−1810.
(25) Mitsikostas, D. D.; Sanchez del Rio, M. Receptor systems mediating *c-fos* expression within trigeminal nucleus caudalis in animal models of migraine. *Brain Res. Rev.* **2001**, *35*, 20−35 and references therein.

JM025548Q