Letters

Two Prodrugs of Potent and Selective GluR5 Kainate Receptor Antagonists Actives in Three Animal Models of Pain

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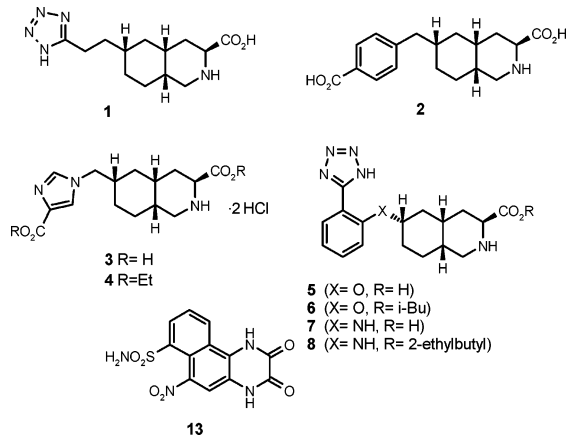
Abstract: Amino acids **5** and **7**, two potent and selective competitive GluR5 KA receptor antagonists, exhibited high GluR5 receptor affinity over other glutamate receptors. Their ester prodrugs **6** and **8** were orally active in three models of pain: reversal of formalin-induced paw licking, carrageenan-induced thermal hyperalgesia, and capsaicin-induced mechanical hyperalgesia.

Glutamate is the primary neurotransmitter in the mammalian central nervous system. Knowledge of the existence of subtypes of glutamate receptors was advanced by the observations that excitatory amino acids (EAA) such as *N*-methyl-D-aspartate (NMDA), quisqualate, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), and kainic acid (KA) showed different potencies on subsets of neurons. This information, coupled with recent molecular biology studies, led to the current classification of glutamate receptors into metabotropic glutamate receptors[1] (G-protein-coupled) and ionotropic glutamate receptors[2] (ligand-gated ion channels). The latter receptors are defined, on the basis of subtype selective agonists, as NMDA, AMPA, and KA. Of the AMPA and KA ionotropic glutamate receptors cloned, subunits GluR1−4 are AMPA-sensitive, while GluR5−7 and KA1−2 are AMPA-insensitive and KA-preferring.[3]

Transfer of nociceptive information from the periphery to the spinal cord occurs via C-fiber primary afferents. Within dorsal roots, dorsal root ganglion (DRG) cell bodies are associated with primary afferent neurons. It was the early work of Agrawal and Evans that identified that C-fiber afferents possess KA receptors.[4] The biophysical and pharmacological profile of KA receptors in DRG neurons suggests that they likely comprise GluR5 homomers.[5] Recent studies in spinal cord slices have implicated KA receptors, specifically the GluR5 receptor subtype, in pain transmission.[6] Sang et al. showed that the decahydroisoquinoline **1** (LY293558),[7] 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.[8] Simmons et al. showed that **2** (LY382884),[9] a selective GluR5 KA receptor antagonist, was efficacious in the formalin test[10] (an animal model of persistent pain).[11] More recently, Filla et al. showed that **3**, a highly selective competitive GluR5 KA receptor antagonist, exhibited high GluR5 receptor affinity and selectivity over other glutamate receptors. Its diethyl ester prodrug **4** was orally active in two animal models of migraine pain (Chart 1).[12]

Chart 1



We describe here the in vitro activity of **5** and **7** as potent and selective GluR5 KA receptor antagonists, and the oral activity of their ester prodrugs **6** and **8**, in three animal models of pain.

The preparation of **5** (LY458545), **6** (LY467711), **7** (LY457691), and **8** (LY525327) is outlined in Scheme 1. All compounds were derived from the ketone intermediate **9**, the synthesis of which has previously been described.[13] Treatment of **9** with TMSI selectively removed the methyl carbamate protecting group, and the resulting amine was protected again as a Boc derivative. Then, successful reduction of the ketone was achieved with sodium borohydride in the presence of cerium chloride, which provided the hydroxy ester **10** with the desired stereochemistry at C-6, as shown in Scheme 1.[7b] The resulting alcohol was converted to **11** under typical Mitsunobu reaction conditions[14] (PPh_3, DEAD). The benzonitrile **11** was converted to the tetrazole by treatment with neat tri-*n*-butyltin azide at

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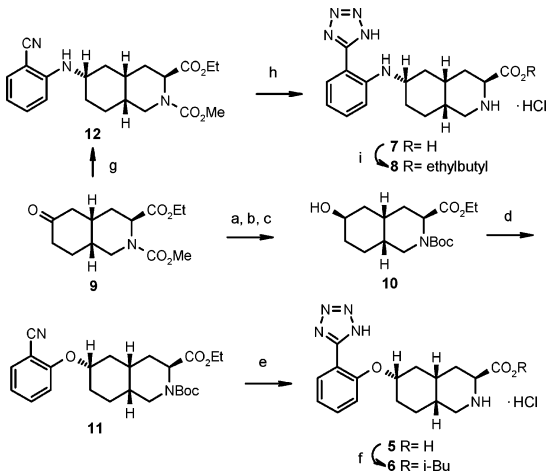
Scheme 1[a]



[a] Conditions: (a) TMSI, CH_2Cl_2, room temp; (b) Boc_2O, Et_3N, CH_2Cl_2, room temp (92% yield two steps); (c) $CeCl_3$, EtOH, $NaBH_4$, from −78 °C to room temp. (60% yield); (d) 2-cyanophenol, PPh_3, DEAD, Py, room temp. (51% yield); (e) (i) n-Bu_3SnN_3, 80 °C; (ii) 2.5M LiOH, THF, 50 °C; (iii) HCl (g)/EtOAc room temp. (50% yield, three steps); (f) i-BuOH/ HCl(g), (92% yield), (g) 2-aminobenzonitrile, $NaBH(OAc)_3$, CH_2Cl_2, room temp. (32% yield); (h) (i) n-Bu_3SnN_3, 80 °C; (ii) 6N HCl reflux (56% yield, two steps); (i) 2-ethylbutyl alcohol/ HCl(g), (87% yield).

80 °C for 3 days followed by ester hydrolysis (2.5 M LiOH), protecting group removal (HCl(g), EtOAc), and purification by ion exchange chromatography to yield the amino acid **5** as a free base (97% purity). Additionally, acid **5** was further esterified to provide ester **6** as the hydrochloride salt (95% purity) under standard conditions with isobutyl alcohol.[15]

Reductive amination of **9** with 2-aminobenzonitrile in the presence of $NaBH(OAc)_3$ provided exclusively **12** in moderate yield. Tetrazole formation and final treatment with 6N HCl and purification by ion exchange chromatography provided the desired amino acid **7** as a free base (98% purity). The amino acid **7** was esterified with 2-ethylbutyl alcohol to yield **8** as a hydrochloride salt (97% purity).

Amino acids **5** and **7** were evaluated in ligand binding studies for their effectiveness in displacing 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 **5** and **7** for the various cloned human AMPA and KA receptors compared with the competitive AMPA/KA antagonist **1**, the selective GluR5 KA receptor antagonist **2**, and the quinoxalidinedione AMPA/KA antagonist **13** (NBQX), a standard tool used in the study of AMPA receptors.[17]

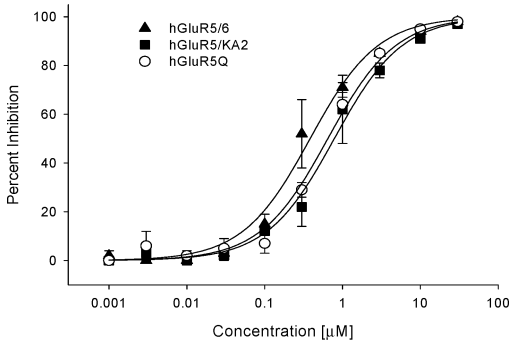


Figure 1. Inhibition of glutamate (100 μM)-invoked calcium influx by **5** in the human GluR5(Q), GluR5/6, and GluR5/KA2 receptors stably transfected in HEK293 cells, with mean value ± SEM for $n = 3$.

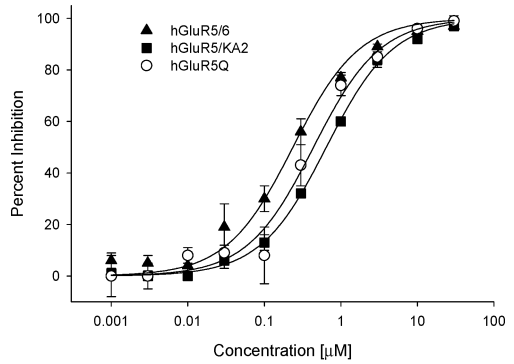


Figure 2. Inhibition of glutamate (100 μM)-invoked calcium influx by **7** in the human GluR5(Q), GluR5/6, and GluR5/KA2 receptors stably transfected in HEK293 cells, with mean value ± SEM for $n = 3$.

Compounds **5** and **7** behaved as antagonists, inhibiting glutamate-evoked calcium influx in the human GluR5(Q), GluR5/6, and GluR5/KA2 receptor stably transfected in HEK293 cell membranes, with IC_{50} values of 0.65 ± 0.10 and 0.44 ± 0.08, 0.38 ± 0.07 and 0.24 ± 0.04, and 0.78 ± 0.01 and 0.65 ± 0.04 μM, respectively (Figures 1 and 2).[16,18]

We evaluated the oral efficacy of these GluR5 KA receptor antagonists in three animal models of pain. Parent amino acids **5** and **7** themselves exhibited poor oral bioavailability in the rat; consequently, we evaluated esters **6** and **8** as potential prodrugs of **5** and **7**, respectively.

Oral dosing of **6** and **8** showed rapid absorption, and their oral bioavailabilities were determined to be 24% and 41%, respectively. Plasma concentrations of **5** and **7** 1 h after dosing following 10 mg/kg oral dose of **6** and **8** were 840 and 938 ng/mL, respectively. On the basis

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

compd	GluR2[b]	GluR5(Q)[c]	GluR5/6[c]	GluR5/KA2[c]	GluR6[c]	GluR6/KA2[c]
1	2.21 ± 0.48	4.16 ± 1.39	2.10 ± 0.76	16.2 ± 4.0	>100	>100
2	>100	17.13 ± 3.60	10.17 ± 6.12	>100	>100	>100
5	8.26 ± 2.03	1.69 ± 0.42	1.00 ± 0.60	7.89 ± 2.16	>100	>100
6	>100	7.37 ± 1.50	5.98 ± 1.14	60.9 ± 22.4	>100	>100
7	5.51 ± 1.76	1.55 ± 0.59	1.37 ± 0.87	9.86 ± 1.93	>100	>100
8	>100	31.75 ± 3.49	13.71 ± 3.63	>100	>100	>100
13	0.21 ± 0.03	11.73 ± 1.74	6.12 ± 2.29	26.6 ± 7.0	13.2 ± 1.9	0.6 ± 0.1

[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. 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.

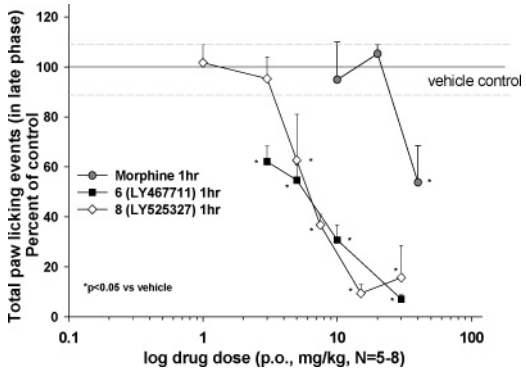


Figure 3. Effects of **6** and **8** on formalin-induced late-phase paw-licking pain behavior 1 h after oral administration in fasted male Sprague Dawley rats. Late phase behavior is defined as 15−40 min after intraplantar injection of 50 μL of 5% formalin in the hind paw. Data are expressed as percent of control (mean ± SEM). Morphine (oral) is plotted as a comparator: (∗) $p < 0.05$ significantly different from vehicle.

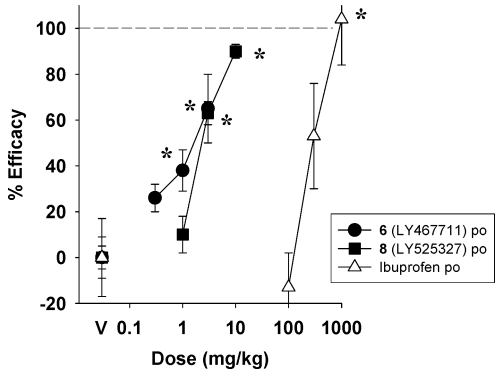


Figure 4. Dose-related reversal of carrageenan-induced thermal hyperalgesia by **6** and **8** in comparison with ibuprofen in rats ($n = 6$ per group).

of these results, we concluded that esters **6** and **8** would act as prodrugs of amino acids **5** and **7**.

In the formalin model, **6** and **8** produced dose-dependent reversal of formalin-induced late-phase paw-licking pain behavior and were highly efficacious after oral administration (Figure 3). Statistically significant effects were evident beginning at 3 mg/kg po with **6** and 5 mg/kg po with **8**. In comparison, morphine was much less potent and efficacious, with a minimal effective dose of 40 mg/kg po (Figure 3).

Both **6** and **8** produced dose-related reversals of carrageenan-induced thermal hyperalgesia with minimal effective doses (MEDs) of 1.0 and 3.0 mg/kg po, respectively (Figure 4). In comparison, the nonsteroidal antiinflammatory drug ibuprofen also reversed carrageenan-induced hyperalgesia with an MED of 1000 mg/kg po.

In addition, **6** and **8** also produced dose-related reversals of capsaicin-induced mechanical hyperalgesia with MEDs of 1.0 and 10 mg/kg po, respectively (Figure 5). Morphine also produced a dose-related reversal of capsaicin-induced hyperalgesia with an MED of 3.0 mg/kg sc.

Compound **6** caused deficits in the rotorod test of neurological function at 30 mg/kg po, whereas **8** showed performance deficits beginning at 20 mg/kg po (Figure 6). Importantly, the effects of **6** and **8** in the persistent

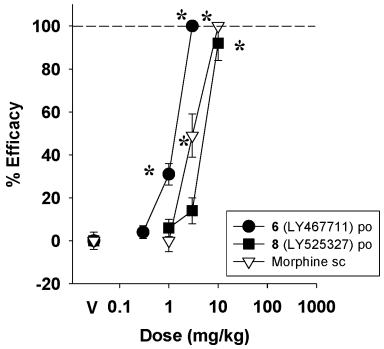


Figure 5. Dose-related reversal of capsaicin-induced mechanical hyperalgesia by **6** and **8** in comparison with morphine in rats ($n = 6$ per group).

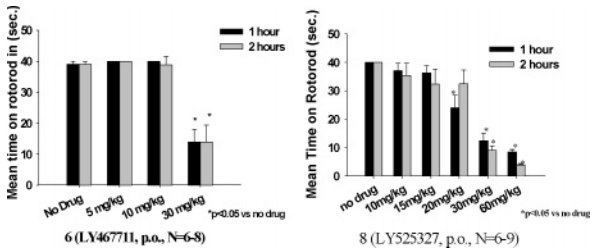


Figure 6. Effects of **6** and **8** on performance on the rotorod 1 and 2 h after oral administration in fasted male Sprague Dawley rats. Baseline performance was 40 s. Data are expressed as time on rotorod in seconds (mean ± SEM): (∗) $p < 0.05$ significantly different from no drug.

pain models occurred at several doses that did not show significant performance deficits in the rotorod test.

In summary, **6** and **8**, the ester prodrugs of amino acids **5** and **7**, demonstrated oral efficacy in three well-established animal models of pain.

Supporting Information Available: Experimental procedures, including analytical and spectral data, for the preparation of **5**−**8** and **10**−**12** and experimental details for the functional studies in recombinant human GluR5 KA receptors and for the three animal models of pain. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Schoepp, D. D.; et al. *Trends Pharmacol. Sci.* **1993**, *14*, 13.
(2) Collingridge, G. L.; Lester, R. A. J. Excitatory amino acid receptors in the vertebrate central nervous system. *Pharmacol. Rev.* **1989**, *40*, 143−210.
(3) (a) Bleakman, D.; Lodge, D. Neuropharmacology of AMPA and KA 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.
(4) Agrawal, S. G.; Evans, R. H. The primary afferent depolarizing action of KA in the rat. *Br. J. Pharmacol.* **1986**, *87*, 345−355.
(5) (a) Huettner, J. E. Glutamate receptor channels in DRG neurons: activation by KA 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 KA receptors by cyclothiazide and concanavalin A. *Neuron* **1993**, *11*, 1069−1082.
(6) (a) Li, P.; Wilding, T. J.; Kim, S. J.; Calejesan, A. A.; Huettner, J. E.; Zhuo, M. Kainate-receptor-mediated sensory synaptic transmission in mammaliam 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 KA 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.

(7) (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-(tetrazolylalkyl)-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.

(8) (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-propionic acid/KA antagonist LY293558 on spontaneous and evoked postoperative pain. *Clin. Pharmacol. Ther.* **2000**, *68*, 320−327.

(9) Bleisch, T.; 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.

(10) Simmons, R. M. A.; Li, D. L.; Hoo, K. H.; Deverill, M.; Ornstein, P. L.; Iyengar, S. Kainate GluR5 receptor subtype mediates the nociceptive reponse to formalin in the rat. *Neuropharmacology* **1998**, *37*, 25−36.

(11) Heapy, C. G.; Jamieson, A.; Russell, N. J. W. Afferent C-fiber and A-delta activity in models of inflammation. *Br. J. Pharmacol.* **1987**, *90*, 164P.

(12) Filla, S. A.; Winter, M. A.; Johnson, K. W.; Bleakman, D.; Bell, M. G.; Bleisch, T. J.; Castaño, A. M.; Clemens-Smith, A.; Del Prado, M.; Dieckman, D. K.; Dominguez, E.; Escribano, A.; Hoo, K. H.; Hudziak, K. J.; Katofiasc, M. A.; Martinez-Perez, J. A.; Mateo, A.; Mathes, B. M.; Mattiuz, E. L.; Ogden, A. M. L.; Phebus, L. A.; Stack, D. R.; Stratford, R. E.; Ornstein, P. L. Ethyl (3*S*,4a*R*,6*S*8a*R*)-6-(4-Ethoxycarbonylimidazol-1-ylmethyl)decahydroisoquinoline-3-carboxylic ester: A prodrug of a GluR5 KA receptor antagonist active in two animal models of acute migraine. *J. Med. Chem.* **2002**, *45*, 4383−4383.

(13) Ornstein, P. L.; Arnold, M. B.; Augenstein, N. K.; Paschal, J. W. Syntheses of 6-oxodecahydroquinoline-3-carboxylates. Useful intermediates for the preparation of conformationally defined excitatory amino acid antagonists. *J. Org. Chem*. **1991**, *56*, 4388−4392

(14) Mitsunobu, O.; Yamada, M. *Bull. Chem. Soc. Jpn.* **1967**, *40*, 2380.

(15) Patent WO 01/02367 A2, 2001 (on prodrugs).

(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 KA 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.

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