Prospects for Metabotropic Glutamate 1 Receptor Antagonists in the Treatment of Neuropathic Pain

Jeffrey M. Schkeryantz,* Ann E. Kingston, and Michael P. Johnson

Lilly Research Labs, Eli Lilly & Co., Lilly Corporate Center, Indianapolis, Indiana 46285

Received August 4, 2006

Introduction

Pain is gaining increasing focus as a major public health problem. Chronic pain affects as many as 70 million people[1] in the U.S. and costs an estimated 70−100 billion dollars per year because of health care costs and lost productivity.[2] Neuropathic pain is a complex, chronic pain state having numerous causal factors afflicting an estimated 4 million people in the U.S., and this number escalates to over 18 million people if one considers neuropathic pain attributed to diabetic complications.[3]

In many cases neuropathic pain is initiated by damage to nerves, but other origins of neuropathic pain include compression of nerve fibers by tumors, scar tissue, and inflamed tissue. Common characteristics of neuropathic pain are chronic hyperalgesia and allodynia.

Hyperalgesia is defined as increasing sensitivity to painful stimuli, while allodynia is described as pain that results from normally nonpainful stimuli. Some of the more common treatments for neuropathic pain include nonpharmacological (surgery, psychotherapy, acupuncture) and pharmacological treatments (anesthetics, opiates, antiepileptics, tricyclic antidepressants, etc.) or more commonly a combination of therapies. Ultimately, the strategy for the pharmalogical treatment of neuropathic pain must take into account the drug's effectiveness, as well as side effects, and perhaps even the cost of treatment.

The excitatory amino acids, including glutamate, are of great physiological importance, playing a role in a variety of neurological, physiological, and psychiatric processes such as synaptic plasticity, motor control, respiration, cardiovascular regulation, sensory perception, and emotional responses. The excitatory amino acid L-glutamate through its many receptors mediates most of the excitatory neurotransmission within the mammalian central nervous system (CNS[a]) and has been implicated in numerous peripheral nervous system pathways. Glutamate is considered to be the major excitatory amino acid in the mammalian brain. To exert its effects, glutamate activates two major receptor classes: the ionotropic glutamate (iGlu) and the metabotropic glutamate (mGlu) receptors. The iGlu receptors are responsible for fast excitatory effects via ligand gated ion channels, while the mGlu receptors typically play a regulatory role on these fast glutamatergic effects by modulating the ion channel activity and/or neurotransmitter release (Figure 1).

The mGluRs belong to the type C class of G-protein-coupled receptors (GPCRs) and are characteristic in that native ligands binds within a pocket formed by the large amino-terminal region.

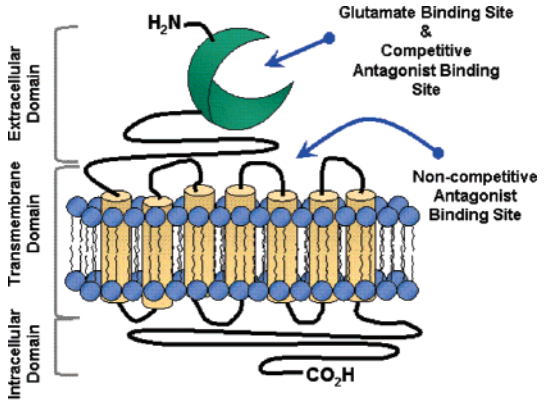


Figure 1. Schematic representation of the mGlu1 receptor (monomeric form).

The calcium-sensing receptors, γ-aminobutyric acid$_B$ (GABA$_B$) receptors, and pheromone receptors also fall within this smaller class of GPCRs.[4] It has been demonstrated that the receptors are localized on presynaptic axonal postsynaptic dendritic and/or glial processes. Here, the mGlu receptors likely modulate neurotransmitter release (either glutamate or other neurotransmitters), postsynaptic membrane excitability, and/or glial/astrocyte function. Currently eight mGlu subtypes have been cloned and are classified into three groups on the basis of their sequence homology, pharmacology, and signal transduction mechanisms.[4] Group I mGlu receptors (mGlu1 and mGlu5) are positively coupled to phospholipase C (PLC) via Gαq-proteins, thereby resulting in the increased hydrolysis of phosphoinositides and intracellular calcium mobilization. The group II mGlu receptors (mGlu2 and mGlu3) negatively coupled to adenylyl cyclase via activation of the Gαi-protein. Similarly, the group III mGlu receptors, including mGlu4, mGlu6, mGlu7, and mGlu8, are negatively coupled to adenylate cyclase via Gαi.

A variety of neurological, psychiatric, and neuroinflammatory disorders, including pain, have been linked to the glutamatergic system through changes in glutamate release.[5] Some of the most compelling evidence for the involvement of mGlu1 receptor in pain is its presence in primary nociceptive afferent nerve terminals.[6] The mGlu1 receptor also maps nicely to many of the important regions of the central nervous system associated with nociceptive processing such as the dorsal root ganglia, the dorsal horn region of the spinal cord, the thalamus, amygdala, and the cerebral cortex.[7] On the basis of their mechanistic relationship to intracellular calcium mobilization and thus neuronal excitability, the inhibition of the group I mGlu receptors, including mGlu1, has been implicated in the treatment of neuropathic pain, thus designating these receptors as interesting drug targets.

* To whom correspondence should be addressed. Phone: (317) 433-5175. Fax: (317) 276-7600. E-mail: jschkaryantz@Lilly.com.

[a] Abbreviations: CNS, central nervous system; mGlu or mGluR, metabotropic glutamate receptor; iGlu, ionotropic glutamate receptor; GPCR, G-protein-coupled receptors; GABA$_B$, γ-aminobutyric acid$_B$ receptor; PLC, phospholipase C; 7TM, seven-transmembrane domain; ECD, extracellular domain; ip, intraperitoneal; CFA, complete Freund's adjuvant; CCI, chronic constriction injury assay.

10.1021/jm060950g CCC: $37.00 © 2007 American Chemical Society
Published on Web 05/10/2007

Structure and Mechanism of the Metabotropic Glutamate Receptors

The mGlu receptors have a seven-transmembrane domain (7TM) consisting of three intracellular and three extracellular loops like other GPCRs. Several splice variants of the mGlu1 receptor are known with the most widely studied including 1a, 1b, and 1d, which show variations in the intracellular carboxy terminus that can regulate receptor coupling specificity and trafficking.[8] The mGlu receptors are unique GPCRs in that their endogenous ligand, glutamate, binds to the extracellur amino terminus portion of the receptor protein translating a signal via the transmembrane segments to the intracellular matrix through receptor/G-protein interactions. The site of action for the endogenous ligand in many of the other GPCRs is their 7TM region. The structure of the extracellular domain (ECD) of mGlu1a has been solved with and without glutamate present, and this has greatly enhanced the mechanistic understanding of glutamate function within the mGlu receptors.[9]

This structure has revealed that the mGlu1a ECD consists of two large globular regions linked by a hinge domain. The agonist binds within this "Venus flytrap" like region and is postulated to stabilize the closed or "active" form of the receptor through positive interactions with both globular regions.[8] In contrast, a competitive antagonist would presumably destabilize this closed form, keeping the ECD in its open form (inactive state) and thus preventing activation of the receptor. Interestingly, the solved structure of the mGlu1a ECD is homodimeric whether the agonist is bound or unbound. In both instances the ECD is linked through a disulfide bond via Cys140.[10] The difference in tertiary structure between the two instances (bound and unbound agonist) resides in the proximal distance between the C-termini of the respective ECDs, which are normally connected to the 7TM. With agonist bound, the C-terminus ends of the two ECDs shift 25 Å closer to each other compared to a homodimer with unbound agonist. This large conformational change upon agonist binding is postulated as the switch that activates the dimeric receptors.[11] A similar model has also been used to explain receptor activation in the $GABA_B$ receptor.[12]

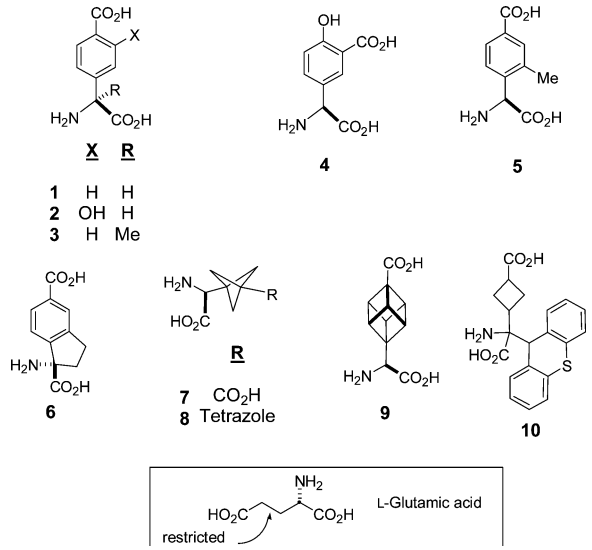
Pharmacological Evidence for mGlu1 Antagonists and Neuropathic Pain

The participation of mGlu1 receptors in chronic or neuropathic pain appears to be well documented.[13,14] Glutamate, released from primary afferent neurons, may play a key role in persistant activation of spinal neurons and hypersensitivity to painful stimuli.[14] In addition, both mRNA and proteins for mGlu1 receptors have been located at spinal, supraspinal, and peripheral sites known to be involved in nociceptive transmission indicating that mGlu1 can modulate nociception at different levels of the nervous system.[15] The intrathecal injection of group I mGlu receptor agonists has been shown to induce spontaneous pain behavior that can be attenuated upon administration of a selective mGlu1 antagonist.[16] Moreover, reports indicate that intrathecal administration of selective mGlu1 receptor antibodies reduces cold hyperalgesia and mechanical allodynia associated with chronic nerve constriction in rats.[17] Finally, heat-related hyperalgesia and mechanical allodynia following chronic constrictive injury of the sciatic nerve have been reduced using antisense oligonucleotide knockdown of spinal mGlu1 receptors.[18]

Competitive Antagonists

The first reported small-molecule mGlu1 antagonists were competitive inhibitors that bind in the extracellular domain.

Chart 1. Competitive mGlu1 Antagonists



Many of these early compounds were derivatives of phenylglycine such as **1** (S-4CPG), **2** (S-MCPG), and **3** (S-4C3HPG) (Chart 1) and as such are some of the most widely studied.[19] The 4-carboxylate analogue (**1**) possesses an IC_{50} of 10−100 μM. Interestingly, both weak agonist as well as weak antagonist activity for this compound has been reported. This compound exhibited no activity on the group III receptor, mGlu4. The structurally related compound **2** introduces a hydroxyl group ortho to the carboxylate group in compound **1**. Compound **2** is a competitive antagonist at the mGlu1 receptor ($IC_{50} = 40\ \mu$M) with mGlu2 agonist activity ($EC_{50} = 48\ \mu$M). The phenylglycine derivative with an α-methyl substituent (**3**) is endowed with both mGlu1 and mGlu2 antagonist activity ($IC_{50} \approx 4.3\ \mu$M). Although more potent than compound **1**, the identification of mGlu8 antagonistic effects in compound **3** has reduced its usefulness as a tool for studying mGlu1 antagonist pharmacology.[20] Transposition of the carboxylate and the hydroxyl on compound **2** provides compound **4** (S-3-CHPG), which shows weak (∼40 μM) mGlu1 antagonist activity.[19] Incorporating a methyl group ortho to the amino acid functionality in **1** provided **5** (LY367385), a compound with competitive mGlu1 antagonist activity that is also subtype-selective (no activity at mGlu5).[21] This is one of the first reported examples of a subtype-selective small-molecule mGlu1 antagonist and consequently helped form the foundation of small-molecule mGlu1 antagonists playing a role in pain mechanisms. For example, intracerebral injection of **5** reduced noxious excitatory responses in the rat thalamus.

Pellicciari reasoned that a constrained analogue of **1** providing (*RS*)-1-aminoindan-1,5-dicarboxylic acid **6** (AIDA) might shed light on the pharmacological features for potency and selectivity.[22] While **6** turned out to be a less potent mGlu1 receptor antagonist than **1**, it has increased selectivity, with no activity at the group II and group III subtypes. It too is subtype-selective with no apparent activity at mGlu5. As with **5**, dosing **6** via intraspinal injection produced responses suggestive of mGlu1 antagonists as an amenable approach for the relief of pain.[23]

The pharmacophore for competitive mGlu1 receptor antagonist activity has a common theme in conformational restriction of the highlighted bond in glutamate (see Chart 1). The phenylglycine derivatives have utilized a phenyl ring to achieve this restricted rotation. In an extension of this theme Pellicciarri synthesized **7** (*S*-CBPG) and **8** (*S*-TBPG) where the rigid [1.1.1] propellane linker substitutes for the phenyl ring.[24] Compound **9** (ACUDA) employs the cubane linker for a similar purpose.[25]

All three of the compounds were weak competitive antagonists of mGlu1 with IC_{50} values ranging from 25 μM for **7** to 230 μM for **9**. These compounds possessed good selectivity against the other mGlu receptors with very little or no potency at mGlu2, mGlu4, and mGlu5. An analogous approach is illustrated by replacement of the phenylgycine moiety with a cyclobutylglycine group. This conformation change increased antagonist potency at mGluR1 but also reduced selectivity vs mGluR5 and the groups II and III mGlu receptors. The compound (±)-2-amino-2-(3-*cis*- and *trans*-carboxycyclobutyl-3-(9-thioxanthyl)-propionic acid) (**10**) had an mGlu1 antagonist IC_{50} value of 1 μM with an order of potency of mGlu1 > mGlu5 > mGlu2 ≥ mGlu8 > mGlu7 > mGlu4.[26] Compound **10** showed antinociceptive effects when dosed intraperitoneally in a model of visceral pain, again indicating the possibility that competitive "amino acid" based antagonists could be useful tools to probe the role of mGluR1 receptors in pain pathways.[26]

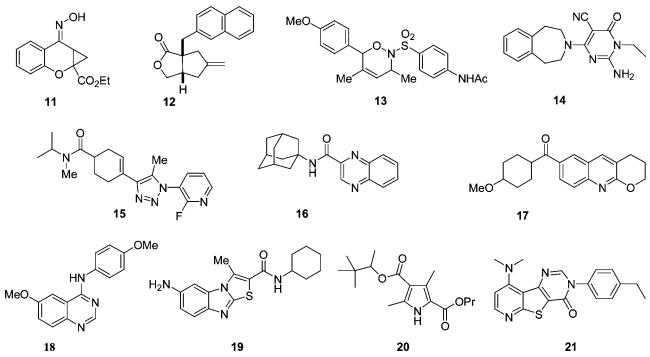
Even though these compounds have functioned as effective tools in elucidating the pharmacology of mGlu1 antagonism, to date the combination of a potent and subtype-selective competitive mGlu1 antagonist has yet to be identified. The difficulty in achieving adequate selectivity is presumably due to the high homology associated with all of the mGlu receptors ECD. The amino acid nature of these molecules also hampers their development into drugs. These extremely polar molecules can be difficult to synthesize and isolate in high enantiomeric purity. These molecules have not developed into drugs in part because of poor plasma exposure and lack of brain penetration, thus precluding their practical use in vivo.

Noncompetitive Antagonists

To circumvent the issues afflicting the competitive ligands, the pharmaceutical industry began searching for novel, non-amino acid ligands to modulate the mGlu receptors in the mid 1990s. This was accomplished by coupling high-throughput screening techniques to functional assays, allowing scientists to identify novel ligands irrespective of their sites of action on the receptor. To date, this method has been used to identify most of the noncompetitive (allosteric) antagonists for the mGlu1 receptor. Whereas the competitive antagonists are rigid derivatives of the amino acid glutamate, the noncompetitive antagonists represent a highly diverse set of chemical entities that have provided additional possibilities for potency, selectivity, and superior physiochemical properties. Through a combination of pharmacology, point mutations, or chimera receptor techniques most of the negative allosteric modulators are believed to bind to the seven-transmembrane domain (7TM) of the mGlu1 receptor, and it is noteworthy that many examples of noncompetitive antagonists display inverse agonist activity, reducing baseline receptor activity, in recombinant mGlu1 cell assays. An increasing number of noncompetitive mGlu1 antagonists are starting to be reported, some with activity in rodent models of pain.

The first noncompetitive (allosteric) antagonist to be reported was 7-hydroxyiminocyclopropan[*b*]chromene-1a-carboxylic acid ethyl ester **11** (CPCCOEt, Chart 2).[27] This chromene-containing molecule antagonized the human mGlu1b receptor with an IC_{50} of 6.6 μM. Compound **12** (BAY36-7620), a lactone derivative disclosed by Bayer, is another more potent allosteric antagonist (IC_{50} = 160 nM).[28] Other structural classes that have been claimed as mGlu1 receptor antagonists include the oxazines, i.e., compound **13**, as well as the azepinyl derivatives (compound **14**).[29,30] Compound **15** containing a triazole central ring has shown very good potency at mGlu1 (IC_{50} = 6 nM) and is highly

Chart 2. Noncompetitive mGlu1 Antagonists



selective over the other mGlu receptors.[31] Not all reported allosteric antagonists are subtype-selective. For instance, the adamantylquinoxaline-containing molecules such as **16** (NPS 2390) showed antagonist activity at both mGlu1 and mGlu5 receptors.[32]

Although some of the allosteric antagonists described above have shown in vivo efficacy in animal models of epilepsy and/or anxiety, their antinociceptive effects have not been well characterized. However, the following classes of molecules do show antinocitive effects in rodent models of pain. One of the most potent noncompetitive antagonists reported to date is (3,4-dihydro-2*H*-pyrano[2,3-*b*]quinolin-7-yl)(*cis*-4-methoxycyclohexyl)-methanone **17** (JNJ16259685).[33] Compound **17** exhibited an excellent functional IC_{50} value of 1.21 nM at the human mGlu1 receptor, and its efficacy has been reported in the rat formalin model of hyperalgesia. Unfortunately the route of administration or relative efficacy was not described. 4-Methoxyphenyl-6-methoxyquinazolin-4-yl **18** (4MPMQA) is reported to have modest potency as a selective mGlu1 receptor antagonist with an IC_{50} of 96 nM and reverses formalin-induced paw-licking behavior when 7.5 mg/kg was administered intraperitoneally (ip).[34] In addition, **18** has shown a reversal of mechanical allodynia in the Chung model of neuropathic pain at doses of 10 and 30 mg/kg ip. This compares quite favorably in potency to gabapentin in this model of persistent pain. The structurally interesting 6-amino-*N*-cyclohexyl-*N*,3-dimethylthiazolo[3,2-*a*]-benzimidazole-2-carboxamide **19** (YM-298198) is reported to possess an IC_{50} value of 16 nM with no activity at the other metabotropic glutamate receptors.[35] Oral administration (30 mg/kg po) of compound **19** prolonged nociceptive response latency in streptozotocin-induced hyperalgesic mice, while no statistically significant effect was seen at 10 mg/kg. Motor coordination was evaluated using the rotorod technique, and no effect was seen at the 30 mg/kg dose.

Two of the most impressive examples of efficacy in rodent models of pain are described for the 2,4-dicarboxypyrrole ester derivative **20** (PPP-1) and the 1,5,7-triazafluorenone derivative (**21**).[36,37]

The bis-ester **20** is a potent and subtype-selective antagonist (IC_{50} = 16 nM) with efficacy in a number of animal models of pain. It showed activity (ED_{50} = 0.3 mg/kg) in both the early and late phases of the formalin assay in mice after oral administration. Intraperitoneal administration (ED_{50} = 3 mg/kg) produced activity in a rat carrageenan model of inflammatory pain. It should be noted, however, that efficacy in a rat model of inflammatory pain does not necessarily correlate to efficacy in a rat model of neuropathic pain. In a rat chronic constriction injury assay (CCI) model of neuropathic pain, **20** was active when dosed at 10 mg/kg ip. Unfortunately this compound's susceptibility to rat plasma esterases and presum-

ably poor oral exposure prevented oral dosing in the rat.[36] Compound **21** (IC_{50} = 3 nM at mGlu1) was efficacious in several rat models of pain. When dosed ip, it had an ED_{50} of 15 μmol/kg in the late phase of the rat formalin model. Fully efficacious effects were seen with compound **21** upon ip administration in the complete Freund's adjuvant (CFA) induced thermal hyperalgesia test and the carrageenan model of inflammatory pain with ED_{50} values of 15 and 11 μmol/kg. No rotorod effects were seen at doses as high as 300 μmol/kg.[37] It is becoming increasingly clear that allosteric mGluR1 antagonists can modulate nociceptive effects in rodent models of pain.

Opportunities for the Future

The advancement of a selective mGlu1 receptor antagonist into the clinic has yet to be reported. This may be due to the lack of oral activity in rodent models of persistent pain, potentially due to poor pharmcokinetics/dynamics. The competitive antagonists are likely too polar, resulting in poor oral absorption and/or penetration through the blood/brain barrier. But unlike the very polar competitive antagonists, the noncompetitive antagonists reported thus far are probably too lipophilic and likely contain metabolic "hot spots", making them susceptible to first-pass metabolism resulting in low plasma and brain exposure when dosed orally. In the future, a mGlu1 receptor antagonist, whether competitive or noncompetitive at the glutamate site, will require the co-optimization of potency, selectivity, and desirable physiochemical properties to elicit oral efficacy in vivo to become a successful drug candidate.

Conclusion

From the onset of its discovery, the mGlu1 receptor has been postulated to play an important role in nociception processing based on the stoichiometry and location of receptor expression in the mammalian brain, in the spinal cord, and on peripheral nerve endings. Intrathecal injection of group I mGlu agonists were shown to increase the response to noxious stimuli in the dorsal horn. Antibodies and antisense reagents against mGlu1 were then discovered that reduced hyperalgesia and allodynia in rodent models of chronic pain, underscoring the hypothesis that antagonists of the mGlu1 receptor could be developed into useful tools for the treatment of pain. Early research from academia and the pharmaceutical industry led to rigid amino acid derivatives of glutamate as competitive antagonists. Unfortunately these were not developed into successful drug candidates because of their inadequate physiochemical characteristics along with their poor selectivity, potency, and brain penetration. The next advance came from the discovery of the noncompetitive antagonists. While noncompetitive mGlu1 antagonists have not been developed into drug candidates for the treatment of pain, they have further validated antagonists of the mGlu1 receptor as attractive drug targets. Fortunately, the large array of structural diversity found in the noncompetitive mGlu1 antagonists provides optimism that the proper balance of in vitro and in vivo antinociceptive activity can be identified, which bodes well for a drug candidate to emerge via this interesting and potentially beneficial target for persistent pain.

Biographies

Jeffrey M. Schkeryantz received his B.S. degree from the University of Wisconsin—Milwaukee in 1987 and his Ph.D. in Synthetic Organic Chemistry from the University of Michigan in 1993 under the supervision of Professor William Pearson. After completing a postdoctoral fellowship with Professor Samuel Danishefsky at Yale University and Sloan-Kettering Cancer Institute, he accepted a position at Abbott Laboratories in 1995. In 1999 he moved to Lilly Research Laboratories and his current title is Head—Discovery Chemistry Research.

Ann E. Kingston received her Ph.D. in Biochemistry from the University of Sussex, U.K., working in the laboratory of Professor John E. Kay. After postdoctoral work at the National Institute for Medical Research, London, she joined Eli Lilly (Lilly Research Center, U.K.) in 1989. Currently, she is a Research Advisor in the Neurodegeneration Team within Neuroscience Discovery at Lilly Research Laboratories in Indianapolis, IN.

Michael P. Johnson received his Ph.D. in Pharmacology and Toxicology from the Purdue University School of Pharmacy in 1991 under the direction of Dr. David E. Nichols. After continuing his education as a postdoctoral fellow with Dr. David Nelson at Lilly Research Laboratories, he accepted a position at Hoechst Marion Roussel (Aventis) in the Neuroscience Discovery Department. In 1997, Dr. Johnson joined Lilly Research Laboratories in the Neuroscience Division and currently is a Research Advisor in the Pain/Migraine Team within Neuroscience Discovery, Lilly Research Laboratories, Eli Lilly & Co., Indianapolis, IN.

References

(1) Krames, E. Intraspinal opioid therapy for chronic nonmalignant pain: Current practice and clinical guidelines. *J. Pain Symptom Manage.* **1996**, *11*, 333−352.

(2) Stewart, W. F.; Ricci, J. A.; Chee, E.; Morganstein, D.; Lipton, R. Lost productive time and cost due to common pain conditions in the U.S. workforce. *JAMA, J. Am. Med. Assoc.* **2003**, *290*, 2443−2454.

(3) Malik, R. A. Can diabetic neuropathy be prevented by angiotensin-converting enzyme inhibitors? *Ann. Med.* **2000**, *32*, 1−5.

(4) For reviews, see the following. (a) Schoepp, D.; Bockaert, J.; Sladeczek, F. Pharmacological and functional characteristics of metabotropic excitatory amino acid receptors. *Trends Pharmacol. Sci.* **1990**, *11*, 1508−1524. (b) Schoepp, D.; Conn, P. J. Metabotropic glutamate receptors in brain function and pathology. *Trends Pharmacol. Sci.* **1993**, *14*, 13−20.

(5) (a) Monaghan, D. T.; Bridges, R. J.; Cotman, C. W. The excitatory amino acid receptors: their classes, pharmacology, and distinct properties in the function of the central nervous system. *Annu. Rev. Pharmacol. Toxicol.* **1989**, *29*, 365−402. (b) Schoepp, D.; Sacaan, A. I. Metabotropic glutamate receptors and neuronal degenerative disorders. *Neurobiol. Aging* **1994**, *15*, 261−263. (c) Meldrum, B.; Garthwaite, J. Excitatory amino acid neurotoxicity and neurodegenerative disease. *Trends Pharmacol. Sci.* **1990**, *11*, 379−387.

(6) (a) Neugebauer, V. Peripheral metabotropic glutamate receptors: fight the pain where it hurts. *Trends Neurosci.* **2001**, *24* (10), 550−552. (b) Neugebauer, V.; Carlton, S. M. Peripheral metabotropic glutamate receptors as drug targets for pain relief. *Expert Opin. Ther. Targets* **2002**, *6* (3), 349−361.

(7) Martin, L. J.; Blackstone, C. D.; Huganir, R. L.; Price, D. L. Cellular localization of a metabotropic glutamate receptor in rat brain. *Neuron* **1992**, *9*, 259−270.

(8) (a) Houamed, K. M.; Kuijper, J. L.; Gilbert, T. L.; Haldeman, B. A.; O'Hara, P. J.; Mulvihill, E. R.; Almers, W.; Hagen, F. S. Cloning, expression, and gene structure of a G protein-coupled glutamate receptor from rat brain. *Science (Washington, D.C.)* **1991**, *252*, 1318−1321. (b) Pin, J-P.; Acher, F. The metabotropic glutamate receptors: structure, activation mechanism and pharmacology. *Curr. Drug Targets: CNS Neurol. Disord.***2002**, *1* (3), 297−317.

(9) (a) Kunishima, N.; Shimadat, Y.; Tsuji, Y.; Sato, T.; Yamamoto, M.; Kumasaka, T.; Nakanishi, S.; Jingami, H.; Morikawa, K. Structural basis of glutamate recognition by a dimeric metabotropic glutamate receptor. *Nature (London)* **2000**, *407*, 971−977. (b) Tsuji, Y.; Shimada, Y.; Takeshita, T.; Kajimura, N.; Nomura, S.; Sekiyama, N.; Otomo, J.; Usukura, J.; Nakanishi, S.; Jingami, H. Cryptic dimer interface and domain organization of the extracellular region of metabotropic glutamate receptor subtype 1. *J. Biol. Chem.* **2000**, *275*, 28144−28151.

(10) Ray, K.; Hauschild, B. C. Cys-140 is critical for metabotropic glutamate receptor-1 dimerization. *J. Biol. Chem.* **2000**, *275*, 34245−34251.

(11) Pin, J.-P.; Galvez, T.; Prezeau, L. Evolution, structure, and activation mechanism of family 3/C G-protein-coupled receptors. *Pharmacol. Ther.* **2003**, *98* (3), 325−354.

(12) Galvez, T.; Duthey, B.; Kniazeff, J.; Blahos, J.; Rovelli, G.; Bettler, B.; Prezeau, L.; Pin, J.-P. Allosteric interactions between GB1 and GB2 subunits are required for optimal GABAB receptor function. *EMBO J.* **2004**, *20* (9), 2152−2159.
(13) Coderre, T. J.; Katz, J.; Vaccarino, A. L.; Melzack, R. Contribution of central neuroplasticity to pathological pain: review of clinical and experimental evidence. *Pain* **1993**, *52* (3), 259−285.
(14) Lesage, A. S. J. Role of group I metabotropic glutamate receptors mGlu1 and mGlu5 in nociceptive signalling. *Curr. Neuropharmacol.* **2004**, *2* (4), 363−393.
(15) Bordi, F.; Ugolini, A. Group I metabotropic glutamate receptors: implications for brain diseases. *Prog. Neurobiol. (Oxford*) **1999**, *59* (1), 55−79.
(16) (a) Fisher, K.; Coderre, T. J. Comparison of nociceptive effects produced by intrathecal administration of mGluR Agonists. *NeuroReport* **1996**, *7*, 2743−2747. (b) Karim, F. W.; Gereau, R. W., IV. Metabotropic glutamate receptor subtypes 1 and 5 are the activators of extracellurar signal-related kinase signaling required for inflammatory pain in mice. *J. Neurosci.* **2001**, *21*, 3771−3779.
(17) Fundytus, M. E.; Fisher, K.; Dray, A.; Henry, J. L.; Coderre, T. J. In vivo antinociceptive activity of anti-rat mGluR1 and mGluR5 antibodies in rats. *NeuroReport* **1998**, *9* (4), 731−735.
(18) (a) Fundytus, M. E.; Yashpal, K.; Chabot, J.-G.; Osborne, M. G.; Lefebvre, C. D.; Dray, A.; Henry, J. L.; Coderre, T. J. Knockdown of spinal metabotropic glutamate receptor 1 (mGluR1) alleviates pain and restores opioid efficacy after nerve injury in rats. *Br. J. Pharmacol.* **2001**, *132*, 354−367. (b) Fundytus, M. E.; Osborne, M. G.; Henry, J. L.; Coderre, T. J.; Dray, A. Antisense oligonucleotide knockdown of mGluR1 alleviates hyperalgesia and allodynia associated with chronic inflammation. *Pharmacol. Biochem. Behav.* **2002**, *73* (2), 401−410.
(19) (a) Schoepp, D. D.; Jane, D. E.; Monn, J. A. Pharmacological agents acting at subtypes of metabotropic glutamate receptors. *Neuropharmacology* **1999**, *38*, 1431−1476. (b) Thomsen, C.; Boel, E.; Suzdak, P. D. Actions of phenylglycine analogs at subtypes of the metabotropic glutamate receptor family. *Eur. J. Pharmacol., Mol. Pharmacol. Sect.* **1994**, *267*, 77−84. (c) Hayashi, Y.; Sekiyama, N.; Nakanishi, S.; Jane, D. E.; Sunter, D. C.; Birse, E. F.; Udvarhelyi, P. M.; Watkins, J. C. Analysis of agonist and antagonist activities of phenylglycine derivatives for different cloned metabotropic glutamate receptor subtypes. *J. Neurosci.* **1994**, *14*, 3370−3377.
(20) (a) Saugstad, J. A.; Kinzie, J. M.; Shinohara, M. M.; Segerson, T. P.; Westbrook, G. L. Cloning and expression of rat metabotropic glutamate receptor 8 reveals a distinct pharmacological profile. *Mol. Pharmacol.* **1997**, *51*, 119−125. (b) Kemp, M. C.; Roberts, P. J.; Pook, P. C.-K.; Jane, D. E.; Jones, A. W.; Jones, P. L.; Sunter, D. C.; Udvarhelyi, P. M.; Watkins, J. C. Antagonism of presynaptically mediated depressant responses and cyclic AMP-coupled metabotropic glutamate receptors. *Eur. J. Pharmacol., Mol. Pharmacol. Sect.* **1994**, *266*, 187−192.
(21) (a) Clark, B. P.; Baker, S. R.; Goldsworthy, J.; Harris, J. R.; Kingston, A. E. (+)-2-Methyl-4-carboxyphenylglycine (LY367385) selectively antagonises metabotropic glutamate mGluR1 receptors. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2777−2780. (b) Kingston, A.; ONeill, M. J.; Bond, A.; Bruno, V.; Battaglia, G.; Nicoletti, F.; Harris, J. R.; Clark, B. P.; Monn, J.; Lodge, D.; Schoepp D. D. Neuroprotective actions of novel and potent ligands of group I and group II. Metabotropic glutamate receptors. *Ann. N.Y. Acad. Sci.* **1999**, *890*, 438−449.
(22) Pellicciari, R.; Luneia, R.; Costantino, G.; Marinozzi, M.; Natalini, B.; Jakobsen, P.; Kanstrup, A.; Lombardi, G.; Moroni, F.; Thomsen, C. 1-Aminoindan-1,5-dicarboxylic acid: a novel antagonist at phospholipase C-linked metabotropic glutamate receptors. *J. Med. Chem.* **1995**, *38*, 3717−19.
(23) Zhang, L.; Lu, Y.; Chen, Y.; Westlund, K. Group I metabotropic glutamate receptor antagonists block secondary thermal hyperalgesia in rats with knee joint inflammation. *J. Pharmacol. Exp. Ther.* **2002**, *300*, 149−156.
(24) (a) Pellicciari, R.; Raimondo, M.; Marinozzi, M.; Natalini, B.; Costantino, G.; Thomsen, C. (*S*)-(+)-2-(3′-Carboxybicyclo[1.1.1]-pentyl)glycine, a structurally new group I metabotropic glutamate receptor antagonist. *J. Med. Chem.* **1996**, *39*, 2874−2876. (b) Costantino, G.; Maltoni, K.; Marinozzi, M.; Camaioni, E.; Prezeau, L.; Pin, J.-P.; Pellicciari, R. Synthesis and biological evaluation of 2-(3′-(1*H*-tetrazol-5-yl)bicyclo[1.1.1]pent-1-yl)glycine (S-TBPG), a novel mGlu1 receptor antagonist. *Bioorg. Med. Chem.* **2001**, *9*, 221−227.
(25) Pellicciari, R.; Costantino, G.; Giovagnoni, E.; Mattoli, L.; Brabet, I.; Pin, J.-P. Synthesis and preliminary evaluation of (*S*)-2-(4′-carboxycubyl)glycine, a new selective mGluR1 antagonist. *Bioorg. Med. Chem.* Letters **1998**, *8*, 1569−1574.
(26) Chen, Y.; Bacon, G.; Sher, E.; Clark, B. P.; Kallman, M. J.; Wright, R. A.; Johnson, B. G.; Schoepp, D. D.; Kingston, A. E. Evaluation of the activity of a novel metabotropic glutamate receptor antagonist (±)-2-amino-2-(3-*cis* and *trans*-carboxycyclobutyl-3-(9-thioxanthyl)-propionic acid) in the in vitro neonatal spinal cord and in an in vivo pain model. *Neuroscience* **1999**, *5*, 787−793.
(27) (a) Annoura, H.; Fukunaga, A.; Uesugi, M.; Tatsuoka, T.; Horikawa, Y. A novel class of antagonists for metabotropic glutamate receptors, 7-(hydroxyimino)cyclopropa[*b*]chromen-1a-carboxylates. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 763−766. (b) Litschig, S.; Gasparini, F.; Rueegg, D.; Stoehr, N.; Flor, P. J.; Vranesic, I.; Prezeau, L.; Pin, J.-P.; Thomsen, C.; Kuhn, R. CPCCOEt, a noncompetitive metabotropic glutamate receptor 1 antagonist, inhibits receptor signaling without affecting glutamate binding. *Mol. Pharmacol.* **1999**, *55*, 453−461. (c) Pagano, A.; Ruegg, D.; Litschig, S.; Stoehr, N.; Stierlin, C.; Heinrich, M.; Floersheim, P.; Prezeau, L.; Carroll, F.; Pin, J.-P.; Cambria, A.; Vranesic, I.; Flor, P. J.; Gasparini, F.; Kuhn, R. The non-competitive antagonists 2-methyl-6-(phenylethynyl)pyridine and 7-hydroxyiminocyclopropan[*b*]chromen-1a-carboxylic acid ethyl ester interact with overlapping binding pockets in the transmembrane region of group I metabotropic glutamate receptors. *J. Biol. Chem.* **2000**, *275*, 33750−33758.
(28) (a) Carroll, F. Y.; Stolle, A.; Beart, P. M.; Voerste, A.; Brabet, I.; Mauler, F.; Joly, C.; Antonicek, H.; Bockaert, J.; Muller, T.; Pin, J.-P.; Prezeau, L. BAY 36-7620: a potent non-competitive mGlu1 receptor antagonist with inverse agonist activity. *Mol. Pharmacol.* **2001**, *59*, 965−973. (b) Stolle, A.; Antonicek, H.-P.; Lensky, S.; Mauler, F.; Mittendorf, J.; Muller, T.; Voerste, A. Synthesis and Structure−Activity-Relationship Studies of Substituted Bicyclic Lactones: A Novel Class of Metabotropic Glutamate Receptor 1 Antagonists. *Abstracts of Papers*, 222nd National Meeting of the American Chemical Society, Chicago, IL, August 26−30, 2001; American Chemical Society: Washington, DC, 2001.
(29) Clark, B. P.; Harris, J. R.; Kingston, A. E. Preparation of 6-Aryl-3,6-dihydro-1,2-oxazines as mGluR1 Receptor Antagonists. WO 2000026199, 2000.
(30) Binggeli, A.; Maerki, H.-P.; Mutel, V.; Wilhelm, M.; Wostl, W. Preparation of tetrahydro-benzo[*d*]azepines-pyrazines and -triazines as metabotropic glutamate receptor antagonists for the treatment or prevention of acute and/or chronic neurological disorders. WO 2002051418, 2002.
(31) Itoh, S.; Satoh, A.; Nagatomi, Y.; Hirata, Y; Suzuki, G.; Kimura, T.; Satow, A.; Hata, M.; Kawamoto, H.; Ohta, H. Discovery and Biological Profile of a New Class of Metabotropic Receptor 1 Allosteric Antagonist. *Abstracts of Papers*, 230th National Meeting of the American Chemical Society, Washington, DC, August 28−September 1, 2005; American Chemical Society: Washington, DC, 2005.
(32) Van Wagenen, B. C.; Moe, S. T.; Smith, D. L.; Sheehan, S. M.; Shcherbakova, I.; Travato, R.; Walton, R.; Barmore, R.; Delmar, E. G.; Stormann, T. M. Preparation of metabotropic glutamate receptor antagonists and their use for treating central nervous system diseases. WO 2000073283, 2000.
(33) Mabire, D.; Coupa, S.; Adelinet, C.; Poncelet, A.; Simonnet, Y.; Venet, M.; Wouters, R.; Lesage, A. S. J.; Van Beijsterveldt, L.; Bischoff, F. Synthesis, structure−activity relationship, and receptor pharmacology of a new series of quinoline derivatives acting as selective, noncompetitive mGlu1 antagonists. *J. Med. Chem.* **2005**, *48*, 2134−2153.
(34) (a) Ambler, S. J.; Baker, S. R.; Clark, B. P.; Coleman, D. S.; Foglesong, R. J.; Goldsworthy, J.; Jagdmann, G. E., Jr.; Johnson, K. W.; Kingston, A. E.; Owton, W. M.; Schoepp, D. D.; Hong, J. E.; Schkeryantz, J. M.; Vannieuwenhze, M. S.; Zia-Ebrahimi, M. S. Preparation of hEterocycles Containing a 4-Substituted Pyrimidine Subunit for Pharmaceutical Use as mGluR1 Antagonists. WO 2001032632, 2001. (b) Schkeryantz, J. M. Novel Series of Potent and Selective Antagonists of mGluR1: Possible Use in the Treatment of Neuropathic Pain. *Abstracts of Papers*, 230th National Meeting of the American Chemical Society, Washington, DC, August 28−September 1, 2005; American Chemical Society: Washington, DC, 2005.
(35) Kohara, A.; Toya, T.; Tamura, S.; Watabiki, T.; Nagakura, Y.; Shitaka, Y.; Hayashibe, S.; Kawabata, S.; Okada, M. Radioligand binding properties and pharmacological characterization of 6-amino-*N*-cyclohexyl-*N*,3-dimethylthiazolo[3,2-*a*]benzimidazole-2-carboxamide (YM-298198), a high-affinity, selective, and noncompetitive antagonist of metabotropic glutamate receptor type 1. *J. Pharmacol. Exp. Ther.* **2005**, *31*, 163−169.
(36) (a) Micheli, F.; Di Fabio, R.; Cavanni, P.; Rimland, J. M.; Capelli, A. M.; Chiamulera, C.; Corsi, M.; Corti, C.; Donati, D.; Feriani, A.; Ferraguti, F.; Maffeis, M.; Missio, A.; Ratti, E.; Paio, A.; Pachera, R.; Quartaroli, M.; Reggiani, A.; Sabbatini, F. M.; Trist, D. G.;

Ugolini, A.; Vitulli, G. Synthesis and pharmacological characterization of 2,4-dicarboxy-pyrroles as selective non-competitive mGluR1 antagonists. *Bioorg. Med. Chem.* **2003**, *11*, 171−183. (b) Micheli, F.; Cavanni, P.; Di Fabio, R.; Marchioro, C.; Donati, D.; Faedo, S.; Maffeis, M.; Sabbatini, F. M.; Tranquillini, M. E. From pyrroles to pyrrolo[1,2-*a*]pyrazinones: a new class of mGluR1 antagonists. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1342−1345.

(37) Zheng, G. Z.; Bhatia, P.; Daanen, J.; Kolasa, T.; Patel, M.; Latshaw, S.; El Kouhen, O. F.; Chang, R.; Uchic, M. E.; Miller, L.; Nakane, M.; Lehto, S. G.; Honore, M. P.; Moreland, R. B.; Brioni, J. D.; Stewart, A. O. Structure−activity relationship of triazafluorenone derivatives as potent and selective mGluR1 antagonists. *J. Med. Chem.* **2005**, *48*, 7374−7388.

JM060950G