Discovery of Allosteric Potentiators for the Metabotropic Glutamate 2 Receptor: Synthesis and Subtype Selectivity of *N*-(4-(2-Methoxyphenoxy)phenyl)-*N*-(2,2,2−trifluoroethylsulfonyl)pyrid-3-ylmethylamine

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Abstract: This report describes recently discovered novel allosteric modulators of metabotropic glutamate2 (mGlu2) receptors. These pyridylmethylsulfonamides (e.g., **3**) potentiate glutamate, shifting agonist potency by 2-fold. This effect was specific for mGlu2 (vs mGlu1,3−8 receptors). Also, **3** failed to potentiate a chimeric mGlu2/1 receptor, demonstrating the mGlu2 transmembrane region's critical involvement. In a fear-potentiated startle model, **3** showed anxiolytic activity that was prevented by mGlu2/3 antagonist pretreatment. Thus, these pyridylmethylsulfonamides represent the first mGlu2 receptor potentiators discovered.

Introduction. Glutamate is the major excitatory neurotransmitter in the central nervous system. Glutamate fast neurotransmission is mediated primarily by the postsynaptic ionotropic glutamate receptors that regulate membrane potential by opening sodium and calcium ion channels. However, glutamate neurotransmission is also mediated by a class of G-protein-coupled receptors (GPCR), the metabotropic glutamate (mGlu) receptors. There are eight subtypes of the mGlu receptors within three groups of mGlu receptors (for a review, see ref 1). The group I receptors, mGlu1 and -5, are primarily considered to be stimulatory, giving rise to postsynaptic increases in cytoplasmic calcium and/or presynaptic increases in neurotransmitter release. In contrast, groups II (mGlu 2 and -3) and III (mGlu4, -6, -7, and -8) are often localized presynaptically and typically inhibit neurotransmission, whether it be on glutamatergic (autoreceptor) synapses or another neurotransmitter-releasing synapse (heteroreceptor function).[2] Our understanding of the physiological importance of these metabotropic GPCRs has been greatly aided by the discovery of pharmacological agents with a degree of specificity for certain mGlu subtypes. One clear example of this is the rigid glutamate analogue 1*S*,2*S*,5*R*,6*S*-2-aminobicyclo[3.1.0]hexane 2,6-dicarboxylate monohydrate (**1**, LY354740), a systemically active group II (mGlu2 and -3) selective agonist.[3,4] Indeed, **1** is often utilized to define the role group II receptors play in behavioral or physiological responses (e.g., refs 5−9). For instance, the efficacy in animal models and more recently clinical trials suggests a mGlu2/3-mediated role in neurological pathways associated with anxiety and/or panic disorders.[3,10−12]

Within the metabotropic glutamate receptor family and especially in a given subgroup, there is a high degree of cloned receptor sequence identity among the glutamate-site binding regions. The high degree of sequence identity with key glutamate-site amino acids might explain the fact that potent mGlu receptor agonists selective for mGlu2 have not been discovered. To identify new structural scaffolds with selectivity for mGlu2 over mGlu3, we initiated the development of a human mGlu2 receptor functional assay to be used for high-throughput screening. The present report describes the resulting discovery and initial characterization of pyridylmethylsulfonamides that are subtype-selective mGlu2 receptor positive allosteric modulators. The structure−activity relationship, in vitro characteristics, and in vivo activity have been briefly described in presentations at the recent Fourth International Meeting of Metabotropic Glutamate Receptors.[13−18]

High-Throughput Screening Functional Assay. Functional assays were developed specifically to allow the discovery of subtype-selective glutamate-site and/or allosteric-site modulators of the mGlu2 receptor. Cell lines expressing the human cDNA sequence of the mGlu receptors[1−8] were generated in AV-12 cells (ATCC CRL-9595) that also expressed the rat glutamate transporter (EAAT1 or glutamate−aspartate transporter) to control extracellular glutamate levels limiting receptor desensitization.[19−21] Additionally, the groups II and III receptor cell lines (mGlu2−4 and mGlu6−8) were engineered to express the promiscuous G protein Gα15.[22,23] This changed the normal coupling of the groups II and III receptors to stimulate intracellular calcium release in a manner similar to that seen with the Gq-coupled group I (mGlu1 and -5) receptors. Thus, transient changes in intracellular calcium levels with agonist addition were measured utilizing Fluo-3 (Molecular Probes, Eugene, OR) and a fluorometric imaging plate reader (FLIPR: Molecular Devices, Sunnyvale, CA) as detailed in the Supporting Information.

Results and Discussion. High-throughput screening of compounds was carried out with a two-addition paradigm. Specifically, test compounds and a submaximally effective (10−20%) concentration (1 μM) of glutamate were simultaneously added to mGlu2 receptor-expressing cells. Thus, the first addition of a positive allosteric modulator or orthosteric agonist increased the transient calcium signal. In high-throughput screening, a second addition of glutamate (90% maximal stimulating) was utilized to identify negative allosteric modulators or orthosteric-site antagonists. In this manner, **2** (*N*-(4-phenoxyphenyl)-*N*-(3-pyridinylmethyl)ethanesulfonamide, LY181837, Scheme 1) was identified as an agent that increased the response of submaximal glutamate.

In follow-up assays, it was found that addition of **1** alone did not significantly alter the response in the mGlu2 expressing cell line. However, when followed with a submaximal glutamate dose, a potentiated response was seen that was similar in magnitude to that of a maximal glutamate dose (Figure 1A). This effect

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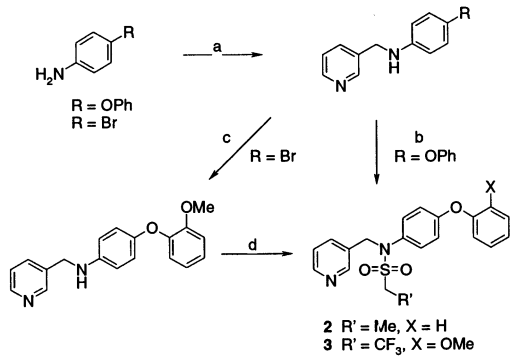
Scheme 1[a]



[a] Reagents and conditions: (a) (i) pyridine-3-carboxaldehyde, MeOH, reflux, (ii) $NaBH_4$, 85%; (b) $EtSO_2Cl$, K_2CO_3, ACN, 54%; (c) guaiacol, K_2CO_3, CuO, pyridine, reflux, 79%; (d) $CF_3CH_2SO_2Cl$, 2:1 1,2-dichloroethane/pyridine, 79%.

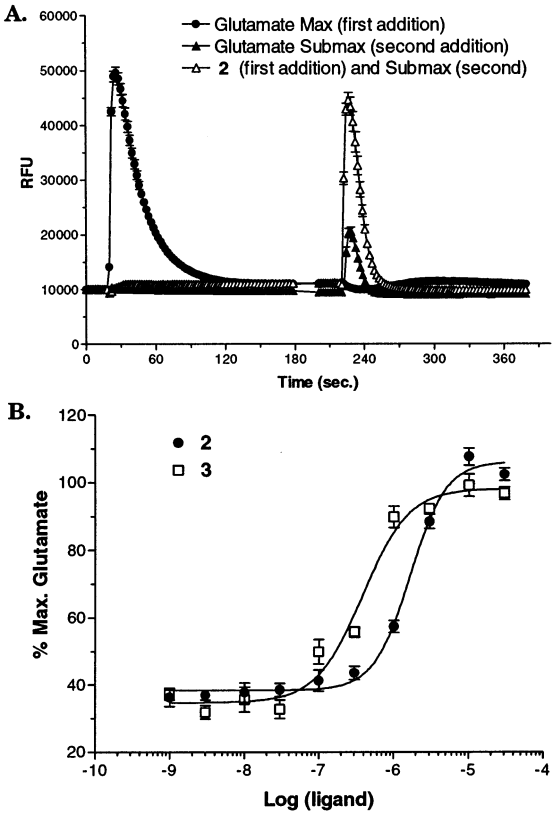


Figure 1. (A) Kinetic tracing of intracellular calcium response in mGlu2 receptor expressing cells. Cells were preloaded with the calcium sensitive dye Fluo-3, and the transient response to the first addition of buffer, 30 μM glutamate (maximal response), or 10 μM **2** after 20 s of baseline measurement was recorded. A second addition of submaximal glutamate (1 μM) resulted in an elevated response in wells containing **2**. (B) Dose−response of varying concentrations of **2** and **3** after addition of 1 μM glutamate. EC_{50} value (mean ± SEM, $n = 3$) for **2** is 1.5 ± 0.1 μM, and for **3** it is 0.27 ± 0.01 μM.

was dose-dependent for **2** with a potentiator EC_{50} of 1.5 ± 0.1 μM (mean ± SEM, $n = 3$, Figure 1B). By use of similar methods, this sulfonamide (**2**) failed to show any indication of significant positive or negative modulatory activity at any of the other seven cloned human mGlu receptors including mGlu3 receptors ($EC_{50} > 12.5$ μM). Similarly, no displacement in standard receptor binding experiment labeling the adrenergic (α1, α2, β), dopamine (D_1, D_2), serotonin (5-HT_2), histamine (H_1), muscarinic, NMDA, AMPA, and kainate receptors was found with **2** (data not shown). Thus, **2** represented a novel compound with a subtype-selective effects at the mGlu2 receptor and a unique mechanism to potentiate the effects of orthosteric agonists such as glutamate.

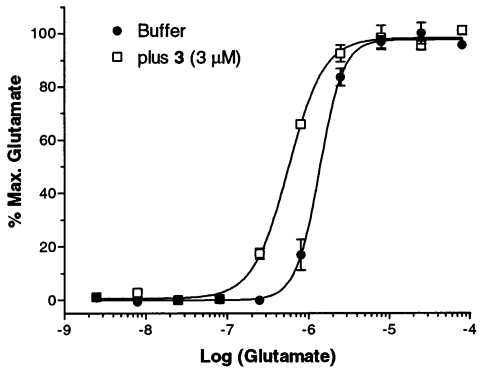


Figure 2. Shift in glutamate dose−response curve with addition of **3** (3 μM). The glutamate EC_{50} value shifted from 1.7 to 0.72 μM with the addition of **3**.

Analogues of **2** were synthesized utilizing methods similar to those for the parent compound (Scheme 1). For example, reductive alkylation of 3-pyridinecarboxaldehyde with 4-phenoxyaniline or 4-bromoaniline afforded the corresponding 4-substituted *N*-(3-pyridylmethyl)anilines. In the latter case, the 4-bromo was replaced with substituted phenols (e.g., guaiacol) to yield various *N*-(4-(substituted phenoxy)phenyl)pridin-3-ylmethylamines. Precursor anilines were acylated with the appropriate sulfonyl chlorides to yield the targeted sulfonamides **2** and **3** (*N*-(4-(2-methoxyphenoxy)phenyl)-*N*-(2,2,2-trifluoroethylsulfonyl)pyrid-3-ylmethylamine, LY487379).

Exploration of the structure−activity relationship in this manner revealed that substitution of a trifluoromethyl- on the terminal carbon side chain of the ethylsulfonamide increased the potency of the potentiator. Furthermore, addition of an ortho methoxy group to the terminal phenyl ring similarly increased potency, resulting in a 5-fold decrease in the EC_{50} value (0.27 ± 0.01 μM, Figure 1B) with **3**. As with **2**, the trifluoroethylsulfonamide **3** did not activate the mGlu2 receptor when given alone but greatly enhanced the response seen with a submaximal glutamate dose (Figure 3). When mGlu2 expressing cells where exposed to **3** (3 μM), the glutamate dose−response curve was shifted parallel to the left, giving a 2-fold decreased glutamate EC_{50} value (0.72 vs 1.7 μM, Figure 2) without significantly altering the maximal response in this cell line. This suggests that these modulators or potentiators act at an allosteric site to increase the affinity of orthosteric agonists. Indeed, **3** failed to displace a glutamate-site antagonist, [3H]-(2*S*,1′*S*,2′*S*)-2-(9-xanthylmethyl)-2-(2′-carboxycyclopropyl)glycine ([3H]-**4**, LY341495) ($IC_{50} >$ 100 μM). Thus, the interaction site of **3** with the mGlu2 receptor does not appear to overlap with the orthosteric-binding site of the antagonist **4**. However, pretreatment with **4** was able to prevent the response seen with **3** in combination with submaximal glutamate (Figure 3B), presumably by limiting glutamate-mediated receptor activation. Thus, these modulators do not bind within the glutamate site or activate the receptor but rather require agonist-induced activation of the mGlu2 receptor

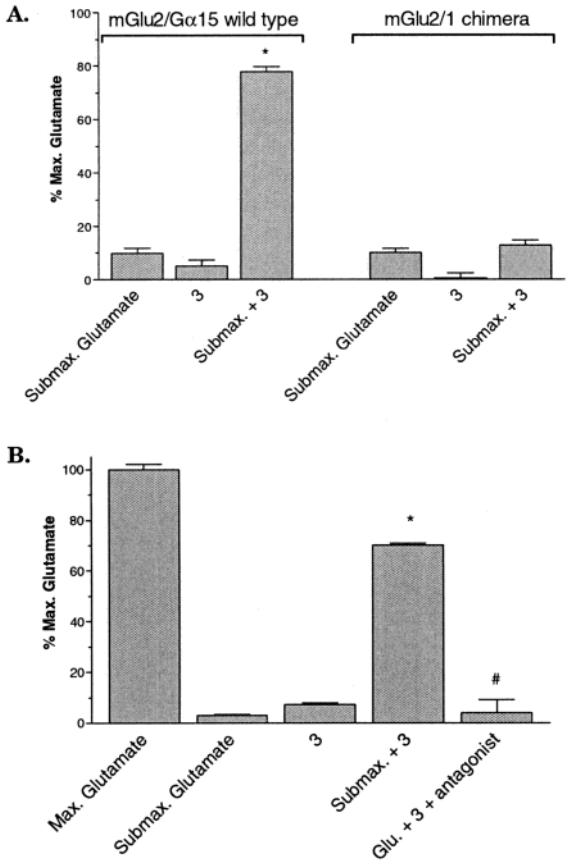


Figure 3. (A) Effect of a submaximal (1 μM) glutamate and **3** (3 μM) alone or in combination on cells expressing either the mGlu2 wild-type or a chimeric mGlu2/1 receptor. The chimera was constructed containing the mGlu2 amino terminus and the mGlu1a transmembrane region, including the extracellular and intracellular loops. (B) Effect of the mGlu2/3 antagonist **4** (3 μM) on the potentiator response by **3** in the mGlu2 expressing cells. Values are expressed as a percent of 100 μM glutamate response (mean ± SEM). The asterisk (∗) indicates significantly greater than submaximal glutamate alone ($p < 0.05$, ANOVA followed by contrast comparison). The pound sign (#) indicates significant difference from the combination of submaximal glutamate and **3** ($p < 0.05$, ANOVA followed by contrast comparison).

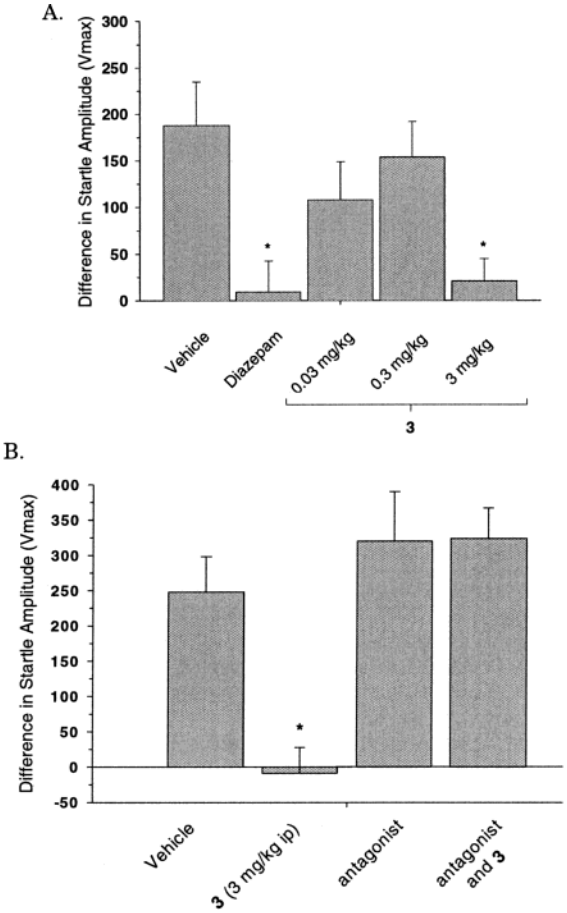


Figure 4. Fear-potentiated startle response in rats after conditioning with foot shock. (A) Diazepam (0.6 mg/kg ip) effectively limited the fear-associated increase in startle response. Similarly, **3** (at 3 but not 0.3 or 0.03 mg/kg ip) limited the fear-associated response. (B) Pretreatment (3 mg/kg sc) of the mGlu2/3-selective antagonists **4** did not significantly alter the fear-potentiated response. However, **4** was able to completely prevent the **3**-mediated decrease in fear-potentiated startle response. The asterisk (∗) indicates significant difference from the vehicle ($p < 0.05$, ANOVA followed by contrast comparison).

for activity. These novel potentiators may act by increasing the sensitivity (or affinity) of the receptor for its neurotransmitter. Alternatively, the potentiators might also act to increase receptor/G-protein coupling. Clarification will have to await a more detailed analysis of the effects of **3** on agonist association, dissociation, and equilibrium binding of agonist radioligands to the mGlu2 receptor.

To further explore the potential allosteric nature of **3**, a chimeric mGlu receptor was constructed by fusing the glutamate site containing the amino-terminal region of the mGlu2 receptor to the transmembrane domain (including the extracellular loops and intracellular domains) of the mGlu1 receptor. As anticipated, when expressed in the AV12/RGT cell line, this chimeric receptor coupled to Gq, resulting in a glutamate-site agonist dependent rise in intracellular calcium and responding to the mGlu2/3 selective agonist (−)-2-oxa-4-aminobicyclo[3.1.0]hexane 4,6-dicarboxylate (**5**, LY379268),[4] with a potency similar to that of the mGlu2 wild type (data not shown). Interestingly, the mGlu2 selective potentiator **3** had no effect on the potency or efficacy of glutamate with this mGlu2/1 chimeric receptor (Figure 3A). Similar results have been obtained with **3**, utilizing mGlu2/3 and mGlu3/2 chimeric receptor constructs (data not shown). Taken together, this clearly illustrates that the key interactions between the potentiators and the mGlu2 receptor are separate and distinct from the orthosteric-site. Furthermore, the results show that the mGlu2 transmembrane domain is critical, suggestive of these potentiators binding within this region of the mGlu2 receptor protein.

To test if these novel allosteric modulators are active in vivo, the effect in a rodent anxiety model (fear-potentiated startle) was examined. Previous work[11] has shown that mGlu2/3 receptor agonists (e.g., **1**) are potent and efficacious in this animal model. This effect can be blocked by pretreatment with an mGlu2/3 selective antagonist. Similarly, a 3 mg/kg ip dose of **3** prevented the shock-conditioned startle magnitude with efficacy equivalent to that of the standard diazepam (Figure 4A). No change in baseline startle was seen with treatment, suggesting that this was not an indirect effect due to sedation or ataxia (data not shown). Furthermore, mGlu2/3 antagonist pretreatment was

able to completely prevent the effect of the mGlu2 potentiator **3** (Figure 4B) but does not alter the response seen with diazepam.[11] Taken together with the in vitro characterization of **3**, the results indicate that this pyridylmethylsulfonamide acts in rats to potentiate a glutamate-mediated stimulation of the mGlu2 receptor following peripheral dosing.

To summarize, the pyridylmethylsulfonamides **2** and **3** are the first reported examples of positive allosteric modulators of the mGlu2 receptor. These compounds are selective for the mGlu2 receptor over the other known mGlu receptors and are unique in that they potentiate without mimicking the effects of orthosteric agonists. Like the previously described mGlu1 and GABAb positive allosteric modulators,[24,25] the sulfonamide potentiators binding domain appears to be separate and distinct from the orthosteric binding site, yet these potentiators increased the potency of agonists such as glutamate in functional assays. Thus, **2** and **3** represent a novel class of allosteric modulators that selectively potentiate the mGlu2 receptor.

Supporting Information Available: Synthetic methods and physicochemical properties of final products and intermediates shown in Scheme 1 and a more detailed description of the calcium assay methods and conditions utilized in binding selectivity assays. This material is available free of charge via the Internet at http://pubs.acs.org.

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