In Vitro and in Vivo Evidence for a Lack of Interaction with Dopamine D\textsubscript{2} Receptors by the Metabotropic Glutamate 2/3 Receptor Agonists 1S,2S,5R,6S-2-Aminobicyclo[3.1.0]hexane-2,6-bicarboxylate Monohydrate (LY354740) and (−)-2-Oxa-4-aminobicyclo[3.1.0] Hexane-4,6-dicarboxylic Acid (LY379268)


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ABSTRACT

Some recently published in vitro studies with two metabotropic glutamate 2/3 receptor (mGluR2/3) agonists ([−(−)]-2-oxa-4-aminobicyclo[3.1.0]hexane-4,6-dicarboxylic acid (LY379268) and 1S,2S,5R,6S-2-aminobicyclo[3.1.0]hexane-2,6-bicarboxylate monohydrate (LY354740)) suggest that these compounds may also directly interact with dopamine (DA) D\textsubscript{2} receptors. The current data fail to show evidence of direct DA D\textsubscript{2} receptor interactions of LY379268 and LY354740 in vitro or in vivo. Instead, these results provide further evidence for a novel antipsychotic mechanism of action for mGluR2/3 agonists.

Glutamate is the primary excitatory neurotransmitter in the mammalian central nervous system, and the variety of its effects in the brain is regulated in part by the diversity of receptor subtypes that respond to glutamate. These receptors include both ionotropic (ligand-gated ion channels) and metabotropic (G protein-coupled) receptor families (Dingle-dine et al., 1999; Schoepf, 2001). Although the ionotropic receptors were the first to be discovered and have been of significant interest as potential therapeutic targets, the metabotropic glutamate (mGlu) receptors are now also of great interest for potential pharmacotherapeutic development. There are eight mGlu receptors, which have been of significant interest as potential therapeutic targets, the metabotropic glutamate (mGlu) receptors are now also of great interest for potential pharmacotherapeutic development.
into three subgroups, group I (mGlu1 and mGlu2), group II (mGlu4 and mGlu5), and group III mGlu (mGlu6/7/8) receptors, on the basis of molecular structure, pharmacology, and signal transduction pathways. The development of selective pharmacological tools for various mGlu receptor subtypes has enabled animal studies suggesting that these receptors may provide potential targets for the treatment of a wide variety of psychiatric and neurological disorders in humans (for reviews see, Schoepf, 2001; Niswender et al., 2005; Pile et al., 2008).

The potential therapeutic promise of mGlu receptor-targeted molecules was recently reinforced by the finding that LY2140023 (the prodrug of the mGlu2/3 agonist LY404039) produced statistically significant improvements in symptoms in a Phase II trial for patients with schizophrenia (Patil et al., 2007). Preclinical data suggest that the antipsychotic actions of mGlu2/3 receptor agonists such as LY404039 are mechanistically distinct from present antipsychotic drugs and mediated via the selective activation of mGlu2 receptors. For example, the ability of mGlu2/3 receptor agonists to attenuate phencyclidine (PCP) and amphetamine (AMP)-evoked hyperactivity in mouse models of psychosis is blocked by the mGlu2/3 receptor antagonist LY341495 (Cartmell et al., 1999; Rorick-Kehn et al., 2007). Studies carried out in transgenic mice reveal that the antipsychotic actions of the mGlu2/3 receptor agonists LY404039 or LY379268 are lost in mice with targeted deletions of mGlu2, mGlu2/3, but not mGlu3 receptors (Patil et al., 2007; Fell et al., 2008; Woolley et al., 2008), whereas antipsychotic drugs (olanzapine, risperidone, and clozapine) block PCP-induced locomotor activity in both the wild-type and mGlu2/3 receptor knockout animals.

However, two different mGlu2/3 receptor agonists, LY354740 and LY379268, were recently suggested to possess partial agonist activity at both the dopamine (DA) D2 long (D2L) and D2 short (D2S) isoforms of the dopamine D2 receptor (Seeman and Guan, 2008; Seeman et al., 2008). The primary evidence used to support this claim was a relatively high affinity for displacing [3H]domperidone binding in either rat striatal tissue or cloned human D2L and D2S receptors, and an apparent partial agonist activity as measured by the stimulation of [35S]GTPγS binding in cells expressing either cloned dopamine D2L or D2S receptors. Kᵦ values for LY379268 at D2 receptors were reported to be in the range of 5 to 30 nM and between 20 and 50 nM for LY354740. Based on the structural similarities of LY404039 to LY379268 and LY354740, Seeman (2008) raised the possibility that the clinical activity of LY2140023 might be due to the actions of LY404039 at D2 receptors rather than mGlu2/3 Receptors.

The findings reported by Seeman and co-workers (2008) of dopamine D2 receptor affinity and partial agonist activity of LY379268 and LY354740 were surprising, because previous studies had not suggested any direct interactions with dopaminergic receptors. Thus, the current work was undertaken as an attempt to replicate the in vitro findings of Seeman and co-workers (2008) and to look for the ability of either LY379268 and LY354740 to 1) displace [3H]domperidone binding to rat striatal tissue or membranes containing human D2L or D2S receptor isoforms and 2) stimulate either D2L or D2S receptor activity as measured by receptor-stimulated binding of [35S]GTPγS in membranes expressing either human isoform. In addition, we conducted a series of behavioral and neurochemical studies that were designed to evaluate the possible interaction of mGlu2/3 receptor agonists with D2 receptors in vivo. Thus, we assessed in vivo striatal D2 receptor occupancy in the rat for LY379268, LY354740 monohydrate, and the partial D2 receptor agonist aripiprazole by using an LC/MS/MS-based method (Barth et al., 2006). Furthermore, we compared LY379268 to the D₂/D₃ antagonist raclopride for reversal of PCP and d-amphetamine-induced hyperlocomotion in wild-type mice or mice deficient of mGlu2/3 receptors or DA D₂ receptors. Finally, brain DA synthesis rate in reserpinized rats (Svensson et al., 1991) was used as a sensitive in vivo neurochemical assay to study potential agonist/antagonist properties of mGlu2/3 receptor agonists at D₂ receptors.

Materials and Methods

In Vitro Experiments. Materials. Domperidone, S-(-)-sulpiride, 3-hydroxytroamin (dopamine), raclopride, 3-(3-hydroxyphenyl)N-propylnperidine (3-PPP), trizma base, N-methyl-d-glucamine (NMDG), and polyethyleneimine were purchased from Sigma-Aldrich (St. Louis, MO). Magnesium chloride hexahydrate, EDTA, and sodium chloride were acquired from Mallinckrodt Chemical (Paris, KY). Aripipazole, LY354740, and LY379268 were synthesized at the Lilly Research Laboratories (Indianapolis, IN). [3H]Domperidone was custom made (50 Ci/mmol) and purchased from PerkinElmer Life and Analytical Sciences (Waltham, MA). [35S]GTPγS (1250 Ci/mmol) was obtained from PerkinElmer Life and Analytical Sciences. Membranes containing human D2 dopamine receptor (short variant, D2S) transfected into Chinese hamster ovary cells were purchased from PerkinElmer Life and Analytical Sciences. Membranes containing human D2 dopamine receptor (long variant, D2L) transfected into Chem-1 cells (Millipore Bioscience Research Reagents, Temecula, CA) were obtained from Millipore Corporation (Billerca, MA). Wheat germ agglutinin PVT SPA beads were purchased from GE Healthcare (Chalfont St. Giles, UK).

[3H]Domperidone Binding Assay. Membrane preparation. Rat striatal samples were purchased from Zivic Laboratories (Pittsburgh, PA). Rat striatal homogenates were prepared as described previously (Wain-Scott et al., 2005). Rat striatal membranes were thawed, diluted to 0.085 mg/tube in binding buffer (13 mM MgCl₂, 1.67 mM EDTA, 133 mM NaCl, and 67 mM Tris-HCl, pH 7.4), and homogenized with a polyclotron. hD2L and hD2S membranes were thawed, diluted to 0.0022 and 0.0085 mg/tube in binding buffer, respectively, and then homogenized with a polyclotron. [3H]Domperidone binding. The domperidone binding assays were adapted from that originally described by Grigoriadis and Seeman (1986). Binding assays were performed in duplicate in 0.4 ml total volume. Membrane suspension (100 μl) and 100 μl of drug dilution were added to 200 μl of binding buffer containing [3H]domperidone (2 nM) in minitubes. Ninitubes were incubated at room temperature for 60 min. The incubation was terminated by rapid filtration through Whatman hydrophilic GF/C, 96-well filter plates that had been presoaked in 0.5% polyethylenimine. The filter plate was washed three times with ice-cold 50 mM Tris-HCl, pH 7.4. Nonspecific binding was defined by sulpiride (10 μM). The amount of [3H]domperidone trapped on the filters was determined by liquid scintillation spectrometry. The actual free radioligand concentration was determined by sampling the supernatant of identical tubes in which the bound radioligand was separated from the free radioligand by centrifugation.

D₂ Receptor-Stimulated [35S]GTPγS Binding Assay. Compounds were diluted in serial half-log dilutions in either deionized H₂O for agonist studies or 1 μM dopamine for antagonist studies. Nonspecific binding was defined in the presence of deionized H₂O for agonist studies or 10 μM raclopride for antagonist studies, with maximal binding defined by 10 μM dopamine for agonist studies and...
1 μM dopamine for antagonist experiments. All tests were performed in duplicate. 

\[^{35}S\]GTP\(\gamma\)S binding was modified from published conditions (Sim et al., 1995; Thomas et al., 1995). Assays were performed in Tris-HCl, MgCl\(_2\), EDTA, and either NaCl or NMDG to enhance partial agonist activity (Lin et al., 2006) with an adaptation to scintillation proximity technology. Final concentrations of each component were 50, 10, and 0.5 mM, respectively, for Tris-HCl, MgCl\(_2\), and EDTA and 100 mM for NaCl or NMDG. \(^{35}S\)GTP\(\gamma\)S was diluted in assay buffer containing GDP, pargyline, and sodium ascorbate (10 μM, 10 μM, and 0.1% \(^{35}S\)GTP\(\gamma\)S (0.2 nM) final concentrations, respectively). Wheat germ agglutinin PVA SPA beads were diluted in assay buffer at 500 μg/25 μl, mixed well, and added to each well at 1 mg bead/well.

Membranes containing human D\(_2\) dopamine receptor (short variant, D\(_{2s}\)) transfected into Chinese hamster ovary cells were thawed quickly, and 1.7 mg of protein were diluted in 20 μl of assay buffer; contents were homogenized and added to the assay plates at 50 μl/well. Membranes containing human D\(_2\) dopamine receptor (long variant, D\(_{2l}\)) transfected into Chem-1 cells (Millipore Bioscience Research Reagents) were thawed, and 3 mg of cells were homogenized in 20 μl of buffer and added to the assay plates at 50 μl/well.

Plates were sealed with clear plate sealers (PerkinElmer Life and Analytical Sciences) and allowed to incubate at room temperature for 2 h before being counted on \(^{35}S\) settings on the PerkinElmer 1450 Microbeta Trilux Scintillation and Luminescence Counter. Data were calculated with Microsoft Excel to convert points to percent-specific binding then analyzed with GraphPad Prism (GraphPad Software Inc., San Diego, CA) by using nonlinear regression analysis to fit curves and generate \(K_{\text{D}}\), \(E_{\text{max}}\), and \(E_{\text{min}}\) values for compounds run in agonist mode and \(I_{\text{C50}}\) and \(E_{\text{max}}\) values for compounds run in antagonist mode.

**In Vivo Experiments.** Animals. All experiments were conducted in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (http://www.nap.edu/readingroom/books/labrats/) and were approved by the Eli Lilly Institutional Animal Care and Use Committee. Eli Lilly is an American Association of Laboratory Animal Care-accredited facility. Male Sprague-Dawley rats (Harlan, Indianapolis, IN) weighing between 200 to 350 g were tested in the receptor occupancy and the dopamine synthesis rate assays. The sample sizes for each experiment were as follows: receptor occupancy, \(n = 4\) to 5 per group; dopamine synthesis rate \(n = 6\) to 12/group. Rats were group housed with standard laboratory chow and water available ad libitum. Rats were maintained on a 12-h light-dark cycle (lights on at 6:00 AM, lights off at 6:00 PM). Male and water available ad libitum. Rats were maintained on a 12-h light-dark cycle (lights on at 6:00 AM, lights off at 6:00 PM). Male and water available ad libitum. Mice used in this study were congenic N5 generated by backcrossing B6 blastocysts, as described previously (Rubinstein et al., 1985), and correctly targeted ES cells were injected into embryonic founders that provided all mGlu2 receptor knockout mice and mGlu3 receptor knockout mice (Taconic Farms) were generated by homologous recombination as described in detail by Fell et al. (2008). The targeting construct was injected into the R1 line of mouse embryonic stem cells. The recombinant embryonic stem cells were injected into murine C57BL/6 blastocysts, and chimeric males were mated with ICR(CD-1) females. Male offspring carrying the null allele were backcrossed for three generations (N3) with ICR(CD-1) females. N3 offspring were then interbred, and siblings homozygous for the null allele were used as founders that provided all mGlu3 receptor knockout mice and mGlu3 receptor knockout mice used here. Males homozygous for the null allele of mGlu3 were intercrossed with females homozygous for the null allele of mGlu3 to produce an intercross colony heterozygous for both mGlu2 and mGlu3 null alleles. Breeding of siblings from the intercross colony produced an intracross colony, and progeny were genotyped to identify homozygotes for both the mGlu2 and mGlu3 null alleles. Founder animals were selected to generate colonies of double knockout mice and wild-type mice that were used in all subsequent experiments.

**Generation of dopamine D\(_2\) receptor knockout mice.** Mice with selective deletions of dopamine D\(_{2l}\) and D\(_{2s}\) receptors (Taconic Farms) were generated by homologous recombination as previously described in Kelly et al. (1997). The D\(_2\) dopamine receptor targeting vector was electroporated into the D3 ES cell line (Doetschman et al., 1985), and correctly targeted ES cells were injected into embryonic day 3.5 B6 blastocysts, as described previously (Rubinstein et al., 1996). Mice used in this study were congenic N5 generated by back-
crossing to the B6 strain for five generations. Wild-type C57BL/6J mice were obtained from Taconic Farms.

Assessment of locomotor behavior. Behaviors were monitored in transparent, plastic shoebox cages with dimensions of 45 × 25 × 20 cm, a 1-cm depth of wood chips as bedding, and a plastic cage top. These items were placed in a rectangular frame containing a grid of 12 photocell beams in an 8 × 4 configuration (Kinder Scientific, Poway, CA) that was positioned 2.5 cm from the floor of the cage for the detection of body movements (ambulations) and recorded by computer for analysis. Mice were initially acclimated to a plastic shoebox cage for 15 min before being given an intraperitoneal injection of LY379268 or raclopride or sterile water. After an additional 30 min, the mice were administered an intraperitoneal injection of sterile water or PCP. Motor activity was monitored for 60 min after the injection of PCP. The same test paradigm was followed for AMP treatment. All drugs were mixed fresh before use and administered intraperitoneally. Mice were dosed with a volume of 10 ml/kg. Software analysis of beam breaks, under the definitions of Hamilton Kinder, resulted in the measurement of three different parameters: ambulations (pattern of beam breaks indicating that the animal has relocated its entire body), distance moved (cm⁻¹), and time at rest (total seconds in a 60-min session in which no new beams were broken, measured at 1-s intervals). The doses of LY379268 (10 mg/kg) and raclopride (3 mg/kg) used in these studies were selected based on the results of preliminary studies carried out in our laboratories. Preliminary dose-response studies revealed that raclopride (1–10 mg/kg) and LY379268 (1–30 mg/kg) significantly reversed PCP (7.5 mg/kg)- and AMP (5 mg/kg)-evoked behavioral activation in C57BL/6J and ICR/CD-1 mice.

Dopamine Synthesis Rate in Reserpined Rats. Eighteen hours before sacrifice, male rats were given vehicle (2 ml/kg sterile saline) or reserpine (5 mg/kg s.c.) and returned to the home cage. The next day, rats were administered vehicle (2 ml/kg sterile saline), 3 mg/kg LY379268, 10 mg/kg LY379268, 10 mg/kg aripiprazole, 30 mg/kg S-(−)-3-PPP, or 1 mg/kg quinpirole. All drugs were administered 30 min before NSD1015 (100 mg/kg s.c.) except aripiprazole, which was dosed 60 min before NSD1015. Thirty minutes after the administration of NSD1015 (100 mg/kg), the animals were moved to an adjacent room before being sacrificed by decapitation and their brains were removed. Each brain was dissected, and the striatum and nucleus accumbens were removed and frozen on dry ice. In a second experiment, we assessed the ability of LY379268 (10 mg/kg) or haloperidol (0.5 mg/kg) to block S-(−)-3-3-PPP (30 mg/kg)-mediated reductions in the dopamine synthesis rate. As found in the previous experiment, male rats were administered 5 mg/kg reserpine s.c. 18 h before sacrifice and returned to the home cage. The next day, rats were dosed with vehicle (2 ml/kg sterile saline), 10 mg/kg LY379268, 0.5 mg/kg haloperidol, 30 mg/kg S-(−)-3-PPP, or the combination of LY379268/S-(−)-3-PPP or haloperidol/S-(−)-3-PPP. All drugs were administered 30 min before NSD1015 (100 mg/kg s.c.). Thirty minutes after the administration of NSD1015 (100 mg/kg), the animals were moved to an adjacent room before being sacrificed by decapitation and the brain was removed for dissection. Each brain was dissected, and the striatum and nucleus accumbens were removed and frozen on dry ice. All drugs were mixed fresh before use and administered subcutaneously and dosed with a volume of 2 ml/kg.

Tissue samples were weighed individually and stored at −80°C in plastic tubes containing 1 ml of radioactivity HCl (0.01 N) and an antioxidant (0.5 mg/ml l-cysteine) until analyzed for DopA levels. Immediately before analysis, samples were thawed at room temperature and ultrasonicated. After sonication, 100 μl of perchloric acid (1.5 M) was added, and the samples were vortexed and stored at 4°C for 60 min. The samples were then centrifuged for 5 min at 12,000 rpm, and the DopA content of the supernatant was analyzed by HPLC with electrochemical detection. A BDS-Hypersil 5-μm C18 analytical column (2 × 150 mm; Thermo Fisher Scientific, Waltham, MA) was used. The mobile phase consisted of 75 mM sodium phosphate monobasic, 50 mg/l 1-octanesulfonic acid sodium salt, 0.5 mM EDTA, and 4% methanol, pH 2.6 (adjusted with phosphoric acid). The flow rate for the analytical column was 0.4 ml/min, which was maintained at 40°C with a column heater. A BAS LC4C electrochemical detector (BAS Bioanalytical Systems, West Lafayette, IN) with a glassy carbon electrode (E = 0.75 V) was used to detect DOPA at a range setting of 5 nA; 20 μl was injected onto the column. The data were collected by using an EZChrom chromatography data system (Scientific Software, San Ramon, CA) running on a Hewlett Packard computer, which calculated peak heights and sample concentrations.

Statistical Analysis. Homologous competition of unlabeled domperidone with [3H]domperidone binding was used for calculation of the Kᵦ and Bmax, using GraphPad Prism, and the IC₅₀ values from competition curves were determined by nonlinear regression analysis with Excel. Mean and S.E.M. were calculated for animals in each dose group. Neurochemical and behavioral data were analyzed by one-way analysis of variance, and then post hoc comparisons were made by a Bonferroni corrected t test.

Results

Radioligand Binding Studies. [3H]Domperidone binding to D₂ receptors has been reported to be able to distinguish agonists from antagonists, based on agonist production of biphasic displacement curves (Seeman et al., 2003). To test whether LY354740 or LY379268 might display similar properties, [3H]domperidone binding was carried out in membranes from rat striatum and in membranes expressing either the cloned human D₂l, or D₂s receptors.

Seeman et al. (2008) have published that LY354740 and LY379268 displaced [3H]domperidone binding in rat striatal membranes in a biphasic manner and that LY379268 also displaced [3H]domperidone binding from membranes expressing the cloned human D₂₅ receptor in a similar fashion. Such displacement is typical of agonists, with the high-affinity component representing the agonist high-affinity state of the receptor, and the lower affinity component representing the agonist low-affinity state. In agreement with a previous report (Seeman et al., 2003), we found that [3H]domperidone binding can reveal biphasic competition curves with the endogenous agonist dopamine (see Supplemental Fig. 1.) However, this phenomenon seemed to be dependent upon the membrane source. Thus, rat striatal membranes showed a robust biphasic displacement curve, the cloned human D₂₅-expressing membranes showed a small biphasic curve, and the D₂₅-expressing membranes showed only a simple monophasic displacement curve to dopamine. In the rat striatal membranes, DA inhibition of [3H]domperidone binding revealed a high-affinity component that constituted approximately 77% of the displaceable binding with an EC₅₀ = 103 nM. In the human D₂₅-expressing membranes, DA also produced a biphasic curve with the high-affinity component accounting for 35% of the displaceable binding and having an EC₅₀ = 118 nM. DA inhibition of [3H]domperidone binding in the D₂₅-expressing membranes gave only a monophasic displacement curve with an EC₅₀ = 8821 nM.

LY354740 and LY379268 were both evaluated for interaction with the [3H]domperidone binding in all three D₂ receptor-expressing membrane preparations. Figures 1 and 2 show the results of examining cloned human D₂₅ and rat striatal membranes, respectively (data with cloned human D₂₅ is shown in Supplemental Fig. 2). As can be seen from these figures, neither LY354740 nor LY379268 produced significant inhibition of binding in any of the membrane prep-
arations up to the highest concentration tested (10 μM). The D2 receptor antagonist/weak partial agonist aripiprazole, run as a control, exhibited the expected high affinity in all three membrane preparations (EC50 = 5.76 nM, D2S; 3.84 nM, D2L; 3.08 nM, rat striatum), completely inhibiting specific [3H]domperidone binding. Dopamine, the endogenous agonist for the receptor, also completely inhibited [3H]domperidone binding with IC50 values dependent upon the membrane preparation examined. Unlabeled domperidone also inhibited [3H]domperidone binding with the expected high affinity. It is interesting to note that unlabeled domperidone was able to inhibit [3H]domperidone binding in striatal membranes to a lower level than the 10 μM sulpiride used to define nonspecific binding (Fig. 2), suggesting that in addition to D2 receptors [3H]domperidone binds to some non-D2 constituent in the striatum.

[35S]GTPγS Binding Studies. To examine the possibility that LY354740 or LY379268 might have functional activity at D2 receptors, the compounds were tested in [35S]GTPγS binding assays by using cloned human D2S and D2L isoforms of the receptor. As controls, the endogenous agonist dopamine and the partial agonist 3-PPP were included in the assays. Aripiprazole, a very weak partial agonist, was also included. Aripiprazole, although having high affinity for D2 receptors, has such low efficacy that only certain in vitro assays are able to reveal its partial agonism (Burris et al., 2002), whereas many assays only see antagonism with aripiprazole (Lawler et al., 1999; Jordan et al., 2007). In addition to standard [35S]GTPγS binding assay conditions, assays were also run in parallel with NMDG replacing sodium, a condition that has been shown to enhance the apparent efficacy of D2 receptor partial agonists in the [35S]GTPγS binding assay (Lin et al., 2006).

Figure 3 shows the effects of the aforementioned compounds on the stimulation of [35S]GTPγS binding in cells expressing the cloned human D2L receptor. DA potently stimulated [35S]GTPγS binding through the D2L isoform (EC50 = 149 nM, NaCl; EC50 = 22.2 nM, NMDG). 3-PPP acted as a partial agonist in assays containing NaCl (Emax = 36.3% relative to DA; Fig. 3A), and efficacy was increased when NMDG was substituted for NaCl (Emax = 74.3% relative to DA; Fig. 3B). In this clone-expressing D2L, there was no detectable agonist activity of aripiprazole, LY379268, or LY354740 regardless of the assay conditions.

In the presence of 1 μM DA to activate D2L-mediated [35S]GTPγS binding, both raclopride and aripiprazole acted as potent full antagonists (Fig. 4) regardless of the buffer conditions. In the presence of NaCl 3-PPP showed some weak antagonist activity (Fig. 4A), which was not seen in the

Fig. 1. [3H]Domperidone binding to human-cloned D2L receptors. Points on the curves represent the mean ± S.E.M. n = 5. The curves for inhibition of [3H]domperidone binding were evaluated by nonlinear regression (GraphPad Prism) using a sigmoidal curve model with a variable slope. The curve fits yielded the following IC50 values: aripiprazole IC50 = 3.84 nM, dopamine IC50 = 3.52 nM, and domperidone IC50 = 8441 nM. For LY354740 and LY379268, there was insufficient inhibition of [3H]domperidone binding to allow curve fitting.

Fig. 2. [3H]Domperidone binding to rat striatal membranes (n = 5). Points on the curves represent the mean ± S.E.M. for five separate experiments. The curves for inhibition of [3H]domperidone binding were evaluated by nonlinear regression (GraphPad Prism) using a sigmoidal curve model with a variable slope. The curve fits yielded the following IC50 values: aripiprazole IC50 = 3.08 nM, dopamine IC50 = 27.6 nM, and domperidone IC50 = 6.95 nM. For LY353740 and LY379268, there was insufficient inhibition of [3H]domperidone binding to allow curve fitting.

Fig. 3. Stimulation of [35S]GTPγS binding by activation of the cloned human D2L receptor in the presence of NaCl (A) or NMDG (B). Points on the curves represent the mean ± S.E.M. for the number of separate experiments (n) listed in the legend. The curves for the stimulation of [35S]GTPγS binding were evaluated by nonlinear regression (GraphPad Prism) using a sigmoidal curve model with a variable slope.
Lack of D₂ Receptor-Mediated Actions of mGlu₂/₃ Agonists

Fig. 4. Antagonism of dopamine-stimulated [³⁵S]GTPγS binding at the cloned human D₂L receptor in the presence of NaCl (A) or NMDG (B). Points on the curves represent the mean ± S.E.M. for the number of separate experiments (n) listed in the legend. The curves for the stimulation of [³⁵S]GTPγS binding were evaluated by nonlinear regression (GraphPad Prism) using a sigmoidal curve model with a variable slope.

presence of NMDG. Neither LY379268 nor LY354740 showed any effects on DA-stimulated [³⁵S]GTPγS binding in the D₂L-expressing cells.

The compounds were also evaluated at the cloned human D₂S isofor any potential agonist activity (Supplemental Fig. 3). In the presence of 100 nM NaCl or 100 mM NMDG, dopamine showed potent stimulation of [³⁵S]GTPγS binding (EC₅₀ = 123 nM, NaCl; EC₅₀ = 35.7 nM, NMDG). The partial D₃ receptor agonist, 3-PPP, stimulated [³⁵S]GTPγS binding to approximately 45% compared with dopamine in the NaCl-containing buffer, whereas aripiprazole, LY379268, and LY354740 showed no agonist activity. When NMDG was substituted for NaCl, the efficacy of 3-PPP increased to approximately 88% compared with dopamine. Aripiprazole showed a slight trend toward agonist activity, but neither LY279368 nor LY354740 showed any agonist stimulation even in the presence of NMDG.

In cloned D₂S receptors, the antagonists aripiprazole and raclopride both potently inhibited the ability of 1 μM DA to stimulate [³⁵S]GTPγS binding in the presence of either NaCl or NMDG in the buffer. Neither 3-PPP, LY379268, nor LY354740 produced measurable antagonism of DA-mediated stimulation of [³⁵S]GTPγS binding in the buffer containing NMDG (Supplemental Fig. 4). In the NaCl-containing buffer, 3-PPP showed a trend toward inhibition of DA-mediated stimulation of [³⁵S]GTPγS binding, with a moderately good fit by nonlinear regression (R² = 0.5153). However, the LY379268 and LY354740 data points in the NaCl-containing buffer showed only poor fits by nonlinear regression (R² = 0.1208 and 0.1914, respectively) with the 95% confidence limits for the curve top estimates overlapping those of the bottom estimates, indicating no significant effect of these compounds on DA-stimulated binding.

**In Vivo D₂ Receptor Occupancy by LC/MS/MS.** The mGlu₂/₃ receptor agonists LY379268 and LY354740 monohydrate did not exhibit significant DA D₂ occupancy in the rat 60 min after intraperitoneal administration (Table 1). However, aripiprazole occupied D₂ receptors in vivo in a dose-dependent fashion after oral gavage (Table 1). High levels of DA D₂ receptor occupancy, 55, 89, and 93%, were achieved after aripiprazole at doses of 10, 30, and 60 mg/kg 90 min after oral administration.

**Effects on LY379268 and Raclopride on PCP or AMP-Induced Hyperlocomotor Activity in mGlu₂/₃ Receptor Knockout Mice.** LY379268 (10 mg/kg) and raclopride (3 mg/kg) were tested for their ability to reverse PCP (7.5 mg/kg) and amphetamine (5 mg/kg)-evoked behaviors in mGlu₂/₃ receptor-deficient mice and their respective wild-type controls. Basal locomotor activity, PCP (7.5 mg/kg), or amphetamine (5 mg/kg)-induced behavioral activation did not show statistically significant differences between the wild-type or mGlu₂/₃ receptor knockout animals (Fig. 5, top). Whereas LY379268 (10 mg/kg) produced a highly significant reversal of PCP (7.5 mg/kg)-induced ambulations in wild-type mice (P < 0.05), the compound was unable to block PCP-evoked increases in amphetamine in amulatory activity in mGlu₂/₃ receptor-deficient mice (Fig. 5, top). The selective dopamine D₃/D₂ receptor antagonist raclopride attenuated PCP-evoked locomotor activation at 3 mg/kg in both the mGlu₂/₃ receptor-deficient mice and their wild-type controls (P < 0.05 in both cases). Pretreatment with the mGlu₂/₃ receptor agonist LY379268 (10 mg/kg) or raclopride (3 mg/kg) significantly blocked amphetamine-induced hyperlocomotion (Fig. 5, bottom) in the wild-type animals (P < 0.05 in both cases). However, LY379268 (10 mg/kg) and raclopride (3 mg/kg) did not reverse AMP (5 mg/kg)-induced behavioral activation.

**TABLE 1**

<table>
<thead>
<tr>
<th>Compound (Route)</th>
<th>Dose</th>
<th>%D₂ RO ± S.E.M.</th>
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<td></td>
<td>mg/kg</td>
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<tr>
<td>LY379268 (i.p.)</td>
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<td>3</td>
<td>2.7 ± 6.6</td>
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<td>10</td>
<td>51 ± 11.7</td>
</tr>
<tr>
<td>Aripiprazole (p.o.)</td>
<td>30</td>
<td>55.5 ± 6.1</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>88.7 ± 1.7</td>
</tr>
<tr>
<td>Haloperidol (p.o.)</td>
<td>0.01</td>
<td>93.1 ± 2.2</td>
</tr>
<tr>
<td></td>
<td>0.03</td>
<td>71.4 ± 1.9</td>
</tr>
<tr>
<td></td>
<td>0.3</td>
<td>86.9 ± 2.0</td>
</tr>
</tbody>
</table>

RO, receptor occupancy.
LY379268 was without effect on amphetamine-induced hyperactivity in the mGlu2/3 receptor-deficient mice. In contrast, raclopride was able to fully block amphetamine-induced hyperactivity in mGlu2/3 receptor-deficient mice (P < 0.05).

**Effects on LY379268 and DA Agonists on the DA Synthesis Rate in the Striatum and Nucleus Accumbens.** The administration of reserpine (5 mg/kg) to male rats significantly increased the DA synthesis rate, measured in vivo as DOPA accumulation, approximately 2-fold in the striatum (Fig. 7, top) and the nucleus accumbens (Fig. 7, bottom). Consistent with previous reports, the DA agonist quinpirole (1 mg/kg) and the partial DA agonist S-(-)-3-PPP (30 mg/kg) significantly decreased DOPA accumulation in the striatum (79 and 67%, respectively; P < 0.01) and the nucleus accumbens (62 and 53.6%, respectively; P < 0.01). The weak partial DA agonist aripiprazole also significantly decreased DOPA accumulation in both the striatum (31%; P < 0.01) and nucleus accumbens (30%; P < 0.01); however, aripiprazole-
mediated reductions in DOPA accumulation were smaller than those seen with $S(-)-3$-PPP (30 mg/kg) or quinpirole (1 mg/kg). In marked contrast to the full DA or partial DA agonists, the selective mGlu2/3 receptor agonist LY379268 was without effect on DOPA accumulation in either the striatum or nucleus accumbens at 3 or 10 mg/kg.

**Effect of LY379268 or Haloperidol on Partial DA Agonist-Mediated Reductions in the DA Synthesis Rate in the Striatum and Nucleus Accumbens.** Figure 8 shows the effect of haloperidol (0.5 mg/kg) or LY379268 (10 mg/kg) on $S(-)-3$-PPP-mediated reductions in DOPA content. As in the previous study, the administration of $S(-)-3$-PPP (30 mg/kg) significantly reduced striatal (Fig. 8, top) and nucleus accumbens (Fig. 8, bottom) DOPA accumulation in reserpinized rats. This reduction was significantly blocked by the coadministration of the selective mGlu$_{2/3}$ receptor agonist LY379268 (10 mg/kg).

**Discussion**

The goal of the present work was to understand the interaction of LY379268 and LY354740 with dopamine D$_2$ receptors that was reported by Seeman and co-workers (Seeman and Guan, 2008; Seeman et al., 2008). However, regardless of whether we looked at $[^3H]$domperidone binding to native or cloned D$_2$ receptor isoforms or D$_2$-mediated stimulation of $[^35S]$GTP$_{S}$ binding, we were unable to see any evidence for interaction of either LY379268 or LY354740 with the dopamine D$_2$ receptor. Likewise, a subsequent series of neurochemical and behavioral assays focused on D$_2$ receptor-mediated actions failed to reveal evidence for a direct interaction of LY379268 or LY354740 with D$_2$ receptors in vivo.

Seeman et al. (2008) reported that LY354740 and LY379268 inhibited $[^3H]$domperidone binding to D$_2$ receptors in rat stri-
atal membranes and cloned human D_{2L} membranes in a clearly bispacific fashion with the high-affinity component giving the following: LY354740, \( K_i = 24 \text{ nM} \) in rat striatal membranes; LY379268, \( K_i = 21 \text{ nM} \) in rat striatal membranes; and \( K_i = 32 \text{ nM} \) in human D_{2L}-expressing membranes. In the current work, the endogenous agonist DA revealed bispacific competition curves with \(^{[3]H} \text{dopamiderone} \) as the radioligand. Aripiprazole, a very low efficacy D_{2} receptor partial agonist (Jordan et al., 2007), produced monophasic competition curves, showing high affinity in all membrane preparations. However, neither LY379268 nor LY354740 gave significant inhibition of \(^{[3]H} \text{dopamiderone} \) binding in any of these membrane preparations up to the highest concentration tested (10 \( \mu \text{M} \)). It is clear that the \(^{[3]H} \text{dopamiderone} \) binding assay as run in the current work was capable of identifying both agonists and antagonists at various forms of the D_{2} receptor. Yet, no significant effects of either LY379268 or LY354740 on \(^{[3]H} \text{dopamiderone} \) binding were seen.

Seeman et al. (2008) also showed that LY354740 and LY379268 acted as partial agonists for stimulating human D_{2L}-mediated \(^{[35]S} \text{GTP}\gamma \text{S} \) binding (approximately 22\% stimulation relative to DA). As would be expected of a low-efficacy partial agonist, both LY354740 and LY379268 acted as antagonists for inhibiting human D_{2L}-mediated \(^{[35]S} \text{GTP}\gamma \text{S} \) binding that was stimulated by 1 \( \mu \text{M} \) DA: LY354740, \( IC_{50} = 400 \text{ nM}, K_i = 43 \text{ nm} \); LY354740, \( IC_{50} = 120 \text{ nM}, K_i = 27 \text{ nM} \) (Seeman et al., 2008). In our assays of D_{2} receptor-mediated stimulation of \(^{[35]S} \text{GTP}\gamma \text{S} \) binding, the partial agonist 3-PPP was able to stimulate \(^{[35]S} \text{GTP}\gamma \text{S} \) binding in both the cloned human D_{2S} and D_{2L}-expressing membranes. In parallel assays substituting NMDG for NaCl in the assays, a condition reported to enhance partial agonist efficacy in in vitro assays (Lin et al., 2006), an increase in the efficacy of 3-PPP was seen. However, no agonist activity of either LY354740 or LY379268 was observed in either assay condition. It should be pointed out that aripiprazole, which has been reported to be a D_{2} receptor partial agonist with very low efficacy, also showed no significant agonist activity in these assays. Depending on the sensitivity of the assay, aripiprazole is reported as an antagonist or a low-efficacy partial agonist (Lawler et al., 1999; Burris et al., 2002; Jordan et al., 2007) in the literature. Thus, if LY354740 or LY379268 were very low-efficacy partial agonists, on the order of aripiprazole, they would not have been picked up in the \(^{[35]S} \text{GTP}\gamma \text{S} \) binding assays used in the current work. However, if either LY354740 or LY379268 had significant affinity for the D_{2} receptor, they would have been picked up in the antagonist form of the \(^{[35]S} \text{GTP}\gamma \text{S} \) binding assays. Both raclopride and aripiprazole produced clear inhibition of DA-stimulated \(^{[35]S} \text{GTP}\gamma \text{S} \) binding, whereas neither LY354740 nor LY379268 produced any significant inhibition out to the highest concentration tested (10 \( \mu \text{M} \)).

Having failed to replicate the in vitro findings of Seeman and co-workers (2008), we designed a series of neurochemical and behavioral studies to examine the possible direct interactions with D_{2} receptors by mGlu_2/3 receptor agonists in vivo. We used an LC/MS/MS-based receptor occupancy method (Chernet et al., 2005; Barth et al., 2006) to determine the interaction of compounds with the D_{2} receptor in the rat striatum. In agreement with previous studies (Natesan et al., 2006), the partial D_{2} receptor agonist aripiprazole (10–60 \( \mu \text{g/kg} \)) dose-dependently occupied D_{2} receptors in the rat striatum after oral dosing. In marked contrast to aripiprazole, neither LY379269 nor LY354740 demonstrated D_{2} receptor occupancy in the rat striatum.

Activation of presynaptic D_{2} receptors, especially the D_{2L} isoform, is thought to be relevant for locomotor activity (Usiello et al., 2000; Wang et al., 2000; Xu et al., 2002; Lindgren et al., 2003). Mice with a targeted deletion of D_{2L} receptors show reduced levels of locomotor and rearing behaviors and appear to be less sensitive to the locomotor-suppressing and cataleptic effects of D_{2} receptor antagonists (Usiello et al., 2000; Wang et al., 2000; Xu et al., 2002). We investigated the relative contribution of mGlu_2/3 receptor and D_{2} receptors in the efficacy of LY379268 and raclopride in the PCP and AMP-evoked hyperlocomotor models of psychosis. Administration of PCP or AMP increased locomotor activity in wild-type animals, mGlu_{2/3} receptor knockout mice, and D_{2} receptor knockout mice. The mGlu_{2/3} receptor agonist LY379268 blocked both PCP and AMP-induced hyperactivity in wild-type mice and mice lacking D_{2} receptors. However, the efficacy of LY379268 against both psycho-stimulants was lost in mGlu_{2/3} receptor-deficient animals. As expected, the selective D_{3}/D_{2} receptor antagonist raclopride was equally effective at reversing PCP and AMP-induced locomotor effects in both wild-type and mGlu_{2/3} receptor-deficient mice; however, the effects of raclopride were lost in D_{2} receptor knockout mice. These data are in agreement with previous studies in mice demonstrating that the effects of mGlu_{2/3} receptor agonists in psychostimulant models of psychosis are dependent on functional mGlu_{2} receptors (Patil et al., 2007; Fell et al., 2008; Woolley et al., 2008) and not mediated through a direct interaction with D_{2} receptors in vivo.

To further evaluate the direct effects of mGlu_{2/3} receptor agonists at D_{2} receptors, we used a dopamine-depleted (reserpinized) animal preparation, which is a particularly sensitive assay for in vivo D_{2} agonist effects and is also sensitive to partial DA agonists with low intrinsic activity due to the development of supersensitive receptors (or increases in receptor reserve) after prolonged synaptic depletion of DA (Carlsson, 1983; Hjorth et al., 1988). Under these conditions, the mGlu_{2/3} receptor agonist LY379268 was without effect on striatal or accumbens DOPA accumulation at either 3 or 10 \( \mu \text{g/kg} \). In contrast, the partial D_{2} receptor agonists aripiprazole or \( S(-)-3\)-PPP and the full D_{2} agonist quinpirole reduced both striatal and accumbens DOPA accumulation, consistent with their intrinsic activity at the D_{2} receptor (Hjorth et al., 1988; Svensson et al., 1991; Íñiguez et al., 2008). In a second experiment, we assessed the ability of LY379268 or the D_{3}/D_{2} antagonist haloperidol to block partial D_{2} agonist-mediated reductions in DOPA accumulation in reserpinized rats. Whereas haloperidol significantly blocked the effects of the partial agonist \( S(-)-3\)-PPP on DOPA accumulation in the striatum and nucleus accumbens, the mGlu_{2/3} receptor agonist LY379268 was without effect on the reductions in DOPA accumulation. Taken together, these data demonstrate that LY379268 does not have obvious direct agonist (either full or partial agonist) or antagonist effects at the D_{2S} receptor in vivo.

The discrepancies between the findings in the current work and those reported by Seeman and co-workers (Seeman and Guan, 2008; Seeman et al., 2008) are puzzling. In a recent meeting abstract, Zysk et al. (2008) could find no...
interaction of either LY354740 or LY379268 with D2 receptors measured by [3H]raclopride, [3H]-4-propyl-9-hydroxy
naphthoxazine, or [3H]domperidone binding assays. In addition, they saw no functional activity at D2 receptors of either LY354740 or LY379268 as measured by GTP·S or CellKey assays (MDS Analytical Technologies, Concord, ON, Can-
da). In a previous study, Seeman et al. (2005) have reported that other glutamatergic compounds, specifically, PCP, ket-
amine, and dizocilpine (MK-801), show relatively high affinity for D2 receptors when assayed using [3H]domperidone binding and act as full agonists when measured in vitro using a [35S]GTP·S binding assay. However, Jordan et al. (2006) saw no agonist or antagonist activity of phencyclidine, ket-
amine, or dizocilpine at cloned D2 receptors measured by either a [35S]GTP·S binding assay or a calcium flux assay. The reasons for the lack of concordance between the findings by Seeman and co-workers and these other laboratories re-
main to be determined.

In conclusion, the in vitro and in vivo findings presented here clearly demonstrate that mGlu2 receptor agonists of the class represented by LY379268 and LY354740 do not directly interact with D1L or D2R receptors. These findings are consistent with the results of a recent clinical trial (Patil et al., 2007) in which patients receiving the mGlu2 receptor agonist prodrug LY2140023 monohydrate did not develop typical side effects associated with blockade of D2 receptors such as extrapyramidal side effects, dyskinesia, akathisia, or parkinsonian-like effects of hyperprolactinemia. Taken to-
gether, our results provide further evidence for a novel, non-
dopaminergic antipsychotic mechanism of action for mGlu2 receptor agonists.

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References

chotic drugs measured using radiolabeled or nonlabeled raclopride tracer. Life Sci 78:593–602.
Cartmell J, Monn JA, and Schoepp DD (1999) The metabotropic glutamate 2/3 receptor agonists LY354740 and LY379268 selectively attenuate phencyclidine-
Fell MJ, Svensson KA, Johnson BG, and Schoepp DD (2008) Evidence for the role of metabotropic glutamate (mGlu)2 not mGlu3 receptors in the preclinical antipsy-
Grigoriadis D and Seeman P (1986) [3H]Domperidone labels only a single population of receptors which convert from high to low affinity for dopamine in rat brain. Naunyn Schmiedebergs Arch Pharmacol 332:21–25.

Received 20 April 2006; accepted 23 May 2006.

Iniguez SD, Cortez AM, Crawford CA, and McDougall SA (2008) Effects of

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Fell MJ, Svensson KA, Johnson BG, and Schoepp DD (2008) Evidence for the role of metabotropic glutamate (mGlu)2 not mGlu3 receptors in the preclinical antipsy-
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Received 20 April 2006; accepted 23 May 2006.

Iniguez SD, Cortez AM, Crawford CA, and McDougall SA (2008) Effects of

Woolley ML, Pemberton DJ, Bate S, Corti C, and Jones DN (2008) The mGlu2 but not the mGlu3 receptor mediates the actions of the mGluR2/3 agonist, LY379268, in mouse models predictive of antipsychotic activity. *Psychopharmacology (Berl)* 196: 431–440.


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Supplemental Figure 1. Dopamine Inhibition of [3H]Domperidone Binding in Different D2 Receptor-Expressing Membranes. Rat Striatum and Cloned Human D2 Receptors. Points on the curves represent the mean ± SEM for the number of separate experiments (n) listed in the legend. The curves for dopamine inhibition of [3H]domperidone binding were evaluated by nonlinear regression (Graphpad Prizm) for fit by one and two-site models. Curves for both rat striatal and hD2L membranes were fit by a two-site model significantly better than by a one-site model (striatal membranes: IC50 = 103 and 28,500 nM for high and low-affinity components respectively; hD2L: IC50 = 118 and 9,920 nM for high and low-affinity components respectively). In contrast, the curve for the hD2S membranes was best fit by a one-site model (IC50 = 8,820 nM).
Supplemental Data


Title: In vitro and in vivo evidence for a lack of interaction with Dopamine D2 receptors by the mGlu2/3 receptor agonists LY354740 and LY379268

Journal: Journal of Pharmacology and Experimental Therapeutics

Supplemental Figure 2. \[^{3}H\]Dopamine Binding to Human cloned D2S Receptors. Points on the curves represent the mean ± SEM for the number of separate experiments (n) listed in the legend. The curves for inhibition of \[^{3}H\]domperidone binding were evaluated by nonlinear regression (Graphpad Prizm) using a sigmoidal curve model employing a variable slope. The curve fits yielded the following IC\textsubscript{50} values: aripiprazole IC\textsubscript{50} = 5.8 nM, domperidone IC\textsubscript{50} = 4.74 nM, dopamine IC\textsubscript{50} = 8770 nM. For LY353740 and LY379268 there was insufficient inhibition of \[^{3}H\]domperidone binding to allow curve fitting.
Supplemental Data


Title: In vitro and in vivo evidence for a lack of interaction with Dopamine D_2 receptors by the mGlu_2/3 receptor agonists LY354740 and LY379268

Journal: Journal of Pharmacology and Experimental Therapeutics

Supplemental Figure 3. Stimulation of [35S]GTPγS Binding by Activation of the Cloned Human D_2S Receptor. Points on the curves represent the mean ± SEM for the number of separate experiments (n) listed in the legends of each panel. Panel A represents results under the standard [35S]GTPγS binding assay (i.e., in the presence of NaCl), while Panel B represents conditions where NaCl was replaced with NMDG. The curves for the stimulation of [35S]GTPγS binding were evaluated by nonlinear regression (Graphpad Prizm) using a sigmoidal curve model employing a variable slope. In both cases dopamine showed potent stimulation of [35S]GTPγS binding (EC_{50} = 123 nM, NaCl; EC_{50} = 35.7 nM, NMDG). The
Supplemental Data


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partial D2 receptor agonist, 3-PPP stimulated [35S]GTPγS binding to about 45% compared to dopamine in the NaCl-containing buffer (Panel A), while aripiprazole, LY279368, and LY354740 showed no agonist activity. When NMDG was substituted for NaCl (Panel B) the efficacy of 3-PPP increased to approximately 88% compared to dopamine. Aripiprazole showed a slight trend towards agonist activity, but neither LY279368 nor LY354740 showed any agonist stimulation even in the presence of NMDG.
Supplemental Figure 4. Antagonism of Dopamine-Stimulated \[^{35}\text{S}\]GTP\(_\gamma\)S Binding at the Cloned Human D\(_{2S}\) Receptor. Points on the curves represent the mean ± SEM for the number of separate experiments (n) listed in the legend. Panel A represents results under the standard \[^{35}\text{S}\]GTP\(_\gamma\)S binding assay (i.e., in the presence of NaCl), while Panel B represents conditions where NaCl was replaced with NMDG. The curves for the inhibition of \[^{35}\text{S}\]GTP\(_\gamma\)S binding were evaluated by nonlinear regression (Graphpad Prizm) using a sigmoidal curve model employing a variable slope. Neither 3-PPP, LY379268, nor LY354740 produced measurable antagonism of DA-mediated stimulation of \[^{35}\text{S}\]GTP\(_\gamma\)S binding by in the buffer.
containing NMDG (Panel B). In the NaCl-containing buffer, 3-PPP showed a trend towards inhibition of DA-mediated stimulation of $[^{35}\text{S}]$GTP$_7$S binding, with a moderately good fit by nonlinear regression ($R^2 = 0.5153$). However, the LY379268 and LY354740 data points in the NaCl-containing buffer showed only poor fits by nonlinear regression ($R^2 = 0.1208$ and 0.1914, respectively) with the 95% confidence limits for the curve top estimates overlapping those of the bottom estimates, indicating no significant effect of these compounds on DA-stimulated binding.