Pharmacological Characterization of a New, Orally Active and Potent Allosteric Metabotropic Glutamate Receptor 1 Antagonist, 4-[1-(2-Fluoropyridin-3-yl)-5-methyl-1H,2,3-triazol-4-yl]-N-isopropyl-N-methyl-3,6-dihydropyridine-1(2H)-carboxamide (FTIDC)


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ABSTRACT
A highly potent and selective metabotropic glutamate receptor (mGluR) 1 antagonist, 4-[1-(2-fluoropyridin-3-yl)-5-methyl-1H-1,2,3-triazol-4-yl]-N-isopropyl-N-methyl-3,6-dihydropyridine-1(2H)-carboxamide (FTIDC), is described. FTIDC inhibits, with equal potency, L-glutamate-induced intracellular Ca²⁺ mobilization in Chinese hamster ovary cells expressing human, rat, or mouse mGluR1a. The IC₅₀ value of FTIDC is 5.8 nM for human mGluR1a and 6200 nM for human mGluR5. The maximal response in agonist concentration-response curves was reduced in the presence of higher concentrations of FTIDC, suggesting the inhibition in a noncompetitive manner. FTIDC at 10⁻⁶ M showed no agonistic, antagonistic, or positive allosteric modulatory activity toward mGluR2, mGluR4, mGluR6, mGluR7, or mGluR8. FTIDC did not displace [³H]L-ququisulate binding to human mGluR1a, indicating FTIDC is an allosteric antagonist. Studies using chimeric and mutant receptors of mGluR1 showed that transmembrane (TM) domains 4 to 7, especially Phe801 in TM6 and Thr815 in TM7, play pivotal roles in the antagonism of FTIDC. FTIDC inhibited the constitutive activity of mGluR1a, suggesting that FTIDC acts as an inverse agonist of mGluR1a. Intrapерitoneally administered FTIDC inhibited face-washing behavior elicited by a group 1 mGluR agonist, (S)-3,5-dihydroxyphenylglycine in mice at doses that did not produce motor impairment. Oral administration of FTIDC also inhibited the face-washing behavior. FTIDC is a highly potent and selective allosteric mGluR1 antagonist and a compound having oral activity without species differences in its antagonistic activity on recombinant human, mouse, and rat mGluR1. FTIDC could therefore be a valuable tool for elucidating the functions of mGluR1 not only in rodents but also in humans.

Metabotropic glutamate receptors (mGluRs) are G protein-coupled receptors, and they are thought to contribute to the fine-tuning of fast synaptic response, neuronal excitability, and neurotransmitter release. To date, eight mGluR sub-
types (mGluR1–mGluR8) have been cloned and classified into three groups based on sequence homology, pharmacological profile, and signal transduction pathway. Group I mGluRs (mGluR1 and mGluR5) are coupled to phospholipase C and subsequent intracellular calcium release via Gq protein. Group II mGluRs (mGluR2 and mGluR3) and group III mGluRs (mGluR4, mGluR6, mGluR7, and mGluR8) are negatively coupled to adenylate cyclase via Gi protein. Group II mGluRs (mGluR2 and mGluR3) and group III mGluRs (mGluR1 and mGluR5) are coupled to phospholipase D and subsequent intracellular calcium release via Gq protein.

In addition to these classifications, several alternative splice variants of mGluRs have been cloned in human and rodents. In mGluR1, mGluR1a is a splice variant with a long carboxyl-terminal intracellular domain, whereas other splice variants such as mGluR1b and mGluR1d lack the long carboxyl-terminal domain. Among them, mGluR1a as well as mGluR1b are major splice variants of mGluR1 in mammalian brain (Soloviev et al., 1999).

To explore the physiological and/or pharmacological roles of these receptors, the development of pharmacologically selective ligands is desirable, because phenotype analysis of genetically manipulated mice is limited by gene compensation, developmental effects, and variance among strains. However, efforts to date have been unsuccessful in identifying subtype-selective mGluR ligands by traditional binding assays using orthosteric radioligands binding to the 1-glutamate binding site. This is probably due to the fact that the amino acid sequences of L-glutamate binding sites are highly conserved between mGluR1 and mGluR5 (Kunishima et al., 2000). Application of high-throughput functional screening to explore mGluR ligands resulted in identification of subtype-selective compounds (Varney and Suto, 2000). The success of this approach is probably due to functional screenings being able to identify compounds interacting with sites different from the L-glutamate binding site, such as the transmembrane (TM) domain, which is less conserved than the L-glutamate binding site.

Several studies with rodents suggest that blockage of mGluR1 could ameliorate CNS disorders, including pain, neurodegeneration, and psychiatric diseases (Varney and Gereau, 2002; Millan, 2003; Spooren et al., 2003; Palucha and Plic, 2005; Simon and Gorman, 2006; Belozertseva et al., 2007). On the other hand, to date the involvement of mGluR1 in human CNS disorders has not been shown. Therefore, it is important to carry out clinical proof-of-concept studies to understand the functions of mGluR1 in humans. To accomplish this, the development of potent “human” mGluR1 antagonists with brain penetrability and oral activity are desired. BAY36-7620 is a systemically active antagonist toward rat mGluR1, but its activity toward human mGluR1 has not been reported (Carroll et al., 2001). EM-TBPC is a much more potent antagonist toward rat mGluR1 but not toward human mGluR1 (Malherbe et al., 2003). JNJ16259685, LY456236, and LY456066 have potent antagonist activities toward both rat and human mGluR1, although their oral activities have not been described previously (Li et al., 2002; Lavreysen et al., 2004). The recently reported novel mGluR1 antagonist YM-298198 is thought to be a selective mGluR1 antagonist with oral activity while only potency toward rat, but not human, mGluR1 has been reported previously (Kohara et al., 2005). More recently, a new mGluR1 antagonist, A-841720, has been shown to be systemically active in rodent pain models (Zheng et al., 2005; El-Kouhen et al., 2006). However, the antagonistic activity of the compound toward human mGluR1 is 10-fold less potent than that toward rat mGluR1 and its selectivity between human mGluR1 and mGluR5 is not very high (about 30-fold) comparing other known noncompetitive mGluR1 antagonists.

We recently identified 4-(1-aryltriazol-4-yl)-tetrahydropridine-ridines as novel mGluR1 antagonists by random functional screening using CHO cells expressing human mGluR1a. In the course of extensive chemical derivatization of these compounds, we identified a highly potent and selective mGluR1 antagonist, 4-[(2-fluoropyridin-3-yl)-5-methyl-1H-1,2,3- triazol-4-yl]-N-isopropyl-N-methyl-3,6-dihydropryidine-1(2H)-carboxamide (FTIDC). In the present article, we characterize the in vitro pharmacological profile and evaluate the in vivo activity of FTIDC. The results show that FTIDC is a highly potent, selective and allosteric antagonist toward human mGluR1. FTIDC is a compound having oral activity without species differences in its antagonistic activity on recombinant human, mouse, and rat mGluR1. Thus, FTIDC could be a valuable tool for elucidating the functions of mGluR1 across species.

**Materials and Methods**

**Characterization of a Novel mGluR1 Antagonist, FTIDC**

FTIDC was identified in-house (Kawamoto et al., 2006). 1-GLutamate was purchased from Sigma-Aldrich (St. Louis, MO). LY367385, LY341495, L-(+)-2-amino-4-phosphonobutyric acid (L-AP4), (S)-3,5-dihydroxyphenylglycine [(S)-3,5-DHPG], and L-quisquulate were purchased from Tocris Cookson Inc. (Bristol, UK). LY456066, R21427, and BAY36-7620 were synthesized in-house for activity comparison with FTIDC. Myo-[3H]inositol (18 Ci/mmol), [3H]Quisqualate (31–33 Ci/mmol), and [35S]GTPyS (1000 Ci/mmol) were purchased from GE Healthcare (Piscataway, NJ). 1-Proline was purchased from Wako Pure Chemicals (Osaka, Japan). Dialyzed fetal bovine serum, culture media, and other reagents used for cell culture were purchased from Invitrogen (Carlsbad, CA). All other reagents used were of molecular or analytical grade where appropriate.

**Methods**

**Stable Cell Lines.** CHO-dhfr− cells stably expressing human mGluR1a were obtained as described previously by Ohashi et al. (2002). CHO-dhfr− cells stably expressing rat mGluR1a and rat mGluR5 were kindly gifted by Dr. S. Nakanishi (Kyoto University, Kyoto, Japan). These cell lines were cultured in Dulbecco’s modified Eagle’s medium (DMEM) with 10% dialyzed fetal bovine serum, 100 U/ml penicillin, 100 U/ml streptomycin, and 1% proline at 37°C with 5% CO2 in a humidified atmosphere. Mouse mGluR1a cDNA was amplified from mouse brain cDNA (QUICK-Clone cDNA; Clontech, Palo Alto, CA) by the PCR method. The mouse mGluR1a cDNA encoded a peptide sequence identical to mouse mGluR1a, previously reported by Zhu et al. (1999). CHO K1 cells were transfected with mouse mGluR1a cDNA cloned into pcDNA3.1 (Invitrogen) and selected in medium supplemented with 500 μg/ml G-418 (Genetix/Invitrogen). The stable cell lines were isolated and selected by their ability to elicit Ca2+ mobilization following 1-glutamate addition. Human mGluR1b cDNA was obtained by replacing the 3’-coding sequence of human mGluR1a (Ohashi et al., 2002) with human mGluR1b cDNA derived from a human cerebellum cDNA library (Clontech, Palo Alto, CA). CHO-NFAT-bla cells (Invitrogen) were transfected with human mGluR1b cDNA cloned into pcDNA3.1 hyg (Invitrogen) and selected in the medium described above, supplemented with 250 μg/ml hygromycin B and 250 μg/ml zeocin (Invitrogen). The stable cell lines were isolated and selected in the same way as the CHO cells expressing mouse mGluR1a. Rat glutamate/aspar-
tate transporter (rGLAST) cDNA was obtained from rat brain poly A+ RNA (Clonetech) using RT-PCR, and it was cloned into pcDNA3. This rGLAST cDNA encoded a peptide sequence identical to the rGLAST sequence, previously reported by Storck et al. (1992). The CHO cell line expressing human mGluR1b was transfected with rGLAST cDNA cloned into pIRESneo and selected in medium supplemented with 250 μg/ml hygromycin B, 250 μg/ml zeocin, and 500 μg/ml G-418. Human mGluR2 cDNA was obtained from human brain hippocampus poly A+ RNA (Clonetech) using RT-PCR. This cDNA encoded a peptide sequence identical to the human mGluR2 sequence, previously reported by Flor et al. (1995). CHO-dhfr cells stably expressing human mGluR2 were obtained by a minor modification of the method described by Tanabe et al. (1992). In brief, CHO-dhfr cells were transfected with human mGluR2 cDNA cloned into pDKR-dhfr, a kind gift from Dr. S. Nakanishi. The stable cell lines were isolated and selected by their ability to suppress forskolin-induced cAMP formation following L-glutamate addition. The CHO cell line expressing human mGluR2 was transfected with G0/CcDNA cloned into pcDNA3.1hyg and selected in medium supplemented with 200 μg/ml hygromycin B, in the same way as for the CHO cell line expressing mouse mGluR1a. CHO cell lines stably expressing human mGluR4, human mGluR5, human mGluR6, and human mGluR7 were obtained as described previously by O'Brien et al. (2004). Human mGluR8 cDNA was obtained from human retina cDNA (QUICK-Clone cDNA; Clonetech) by PCR. This cDNA encoded a peptide sequence identical to the human mGluR8 sequence reported previously by Wu et al. (1998), except that Asn768 was replaced by Ile. This replacement is due to a single nucleotide polymorphism (rsSNP ID: rs10511433). CHO K1 cells were transfected with human mGluR8 cDNA cloned into pIRESneo. Stable cell lines were isolated and selected by their ability to suppress forskolin-induced cAMP formation following L-AP4 addition to the medium described above, supplemented with 500 μg/ml G-418.

Membrane Preparation. The cells were cultured in the medium described above. The cells were incubated in glutamate/glutamine-free medium the day before harvest, except if the membranes were to be used to evaluate potentiator activity for mGluR4. Confluent cultures were washed with ice-cold phosphate-buffered saline (PBS) and stored at −80°C until membrane preparation. After thawing, cells were suspended in ice-cold buffer A (10 mM MOPS, pH 7.4, 154 mM NaCl, 10 mM KCl, and 0.8 mM CaCl2) containing 20% sucrose, and they were homogenized using a Polytron homogenizer (Kinematica, Luzern, Switzerland). The homogenate was centrifuged at 10,000g for 20 min at 4°C. The supernatant was collected and centrifuged at 100,000g for 60 min at 4°C. The resultant pellet was suspended in buffer B (20 mM HEPES, pH 7.4, and 0.1 mM EDTA) supplemented with protease inhibitor cocktail (Complete EDTA-free; Roche Diagnostics, Mannheim, Germany) and recentrifuged at 100,000g for 60 min at 4°C. The pellet was resuspended in buffer B and stored in aliquots at −80°C until use. Protein content was measured using the bicinchoninic acid method (Sigma-Aldrich) with bovine serum albumin as the standard.

Intracellular Ca2+ Mobilization. CHO cells expressing human mGluR1a were seeded at 5 × 104 cells/well in a 96-well black well/ clear bottom plate (PerkinElmer Life and Analytical Sciences, Boston, MA) and cultured overnight. The cells were then incubated with 4 μM Fluo-3 in assay buffer (Hanks’ balanced salt solution containing 20 mM HEPES and 2.5 mM probenecid) containing 1% dialyzed fetal bovine serum for 1 h at 37°C with 5% CO2 in a humidified atmosphere. The extracellular dye was removed by washing the cells four times with the assay buffer. Ca2+ flux was measured using a fluorometric imaging plate reader (FLIPR, Molecular Devices, Sunnyvale, CA). Cells were pretreated for 5 min with the test compounds to evaluate the agonistic activity of the compounds. After pretreatment, antagonistic activity was evaluated for 3 min after addition of L-glutamate. The final concentration of L-glutamate was 10 μM in the antagonist assay for human mGluR1a and mGluR5. In the antagonist assay for human mGluR1b, mouse mGluR1a, rat mGluR1a, and rat mGluR5, the final concentrations of L-glutamate were 30, 50, and 3 μM, respectively. CHO cells expressing human mGluR5 were seeded at 7.5 × 104 cells/well and loaded with 4 μM Fluo-4.

Production of Inositol Phosphates. Human mGluR1a cDNA and rGLAST cDNA were cloned into pcDNA3. HEK293 cells were seeded at 6 × 106 cells in an 80-cm2 flask and cultured overnight in DMEM with 10% fetal bovine serum, 100 U/ml penicillin, and 100 U/ml streptomycin. The cells were transfected with 8 μg of pcDNA3-human mGluR1a and 4 μg of pcDNA3-rGLAST using Lipofectamine reagent (Invitrogen) according to the manufacturer’s instructions, and then they were cultured overnight. The transfected cells were seeded at 1 × 106 cells/well in a 96-well poly-L-lysine-coated plate and cultured for 6 h. Production of inositol phosphates (IP) was measured by a modification of the method described by Brandish et al. (2003). The cell culture medium was changed to DMEM (Invitrogen) supplemented with 10% dialyzed fetal bovine serum, 2 mM L-glutamine, 100 U/ml penicillin, 100 U/ml streptomycin, and 10 μCi/ml myo-[3H]inositol. Cells were cultured overnight. The cells were rinsed twice with PBS ++ (PBS containing 1.05 mM MgCl2 and 0.9 mM CaCl2) and incubated with PBS ++ containing 1 mM L-glutamate pyruvate transaminase (Sigma-Aldrich) and 2 mM sodium pyruvate for 1 h at 37°C with 5% CO2 in a humidified atmosphere. To evaluate the effect of FTIDC on basal IP production, the cells were incubated for 30 min with the compound in PBS ++ containing 10 mM LiCl, 1 U/ml GPT, and 2 mM sodium pyruvate after rinsing with PBS ++. For the antagonist assay, the cells were incubated with L-glutamate for 30 min after 5-min pretreatment with FTIDC. The reaction was stopped by replacing the incubation medium with 200 μl of 0.1 M formic acid, and then the mixture was centrifuged at 3000 rpm for 5 min at 4°C after incubation for 30 min on ice. The supernatants were loaded onto a 100 μl of AG 1-X8 resin bed (200–400 mesh, formate form; Bio-Rad, Hercules, CA) in a MultiScreen-HTS plate (Millipore Corporation, Bedford, MA). The resin was washed with 5 mM sodium tetraborate dehydrate in 60 mM sodium formate. [3H]Inositol phosphates were eluted with 1 M ammonium formate in 0.1 M formic acid. This eluate is referred to as the IP fraction. The radioactivity in the IP fraction was measured using Tricarb2500 (PerkinElmer Life and Analytical Sciences) after addition of ULTIMA GOLD XR (PerkinElmer Life and Analytical Sciences). Total radioactivity remaining in the cell membrane fraction was determined by solubilizing the membranes with 10% Triton X-100 in 0.1 M NaOH and then measuring radioactivity using TopCount (PerkinElmer Life and Analytical Sciences) after addition of Microscint-40 (PerkinElmer Life and Analytical Sciences). The radioactivity in the IP fraction was normalized to the total amount of radioactivity recovered from the solubilized cellular membranes and the radioactivity of [3H]inositol phosphates.

[35S]GTPγS Binding. [35S]GTPγS binding studies were carried out according to the method described by Ozaki et al. (2000) with a minor modification. In brief, membranes prepared from CHO cell-expressing mGluR2 were incubated with test compounds and 400 pM [35S]GTPγS in 20 mM HEPES, 100 mM NaCl, 10 mM MgCl2, 1 mM EDTA, and 5 μM GDP, pH 7.4, containing 1.5 mg of wheat germ agglutinin-coated SPA beads (GE Healthcare, Little Chalfont, Buckinghamshire, UK) at 25°C for 2 h in the absence or presence of 100 μM L-glutamate. Membrane-bound radioactivity was detected by scintillation proxiometry with TopCount. For the mGluR4 assay, incubation time and concentration of L-glutamate were 1 h and 10 μM, respectively. For the mGluR6 assay, incubation time and concentration of L-glutamate were 1.5 h and 50 μM, respectively. For the mGluR8 assay, incubation time and concentration of L-glutamate were 1 h and 3 μM, respectively. For the mGluR7 assay, membranes were incubated at 37°C for 1.5 h in the absence or presence of 1 mM L-AP4.

[3H]Quisqualate Binding. [3H]Quisqualate binding studies were carried out according to the method described by Ohashi et al. (2002) with a minor modification. In brief, membranes prepared from
CHO cells expressing human mGluR1α were incubated with 50 nM [3H]-quisqualate in the absence or presence of test compounds in 0.2 ml of 50 mM HEPES, pH 7.4, containing 10 mM CaCl₂ at room temperature for 2 h. Nonspecific binding was measured in the presence of 10 μM l-quisqualate. Bound and free radioligand were separated by rapid filtration using UniFilter-96 GFC filter plates and a Filtermate 196 harvester (PerkinElmer Life and Analytical Sciences). Radioactivity trapped on the filter was counted with TopCount after the addition of Microscint-0 (PerkinElmer Life and Analytical Sciences).

Construction and Transfection of Chimeric, Point, and Truncated Mutants of mGluR1. cDNAs encoding chimeric mGluR1(693)5a and mGluR5(680)1a were obtained by a PCR-based overlap extension technique (Horton et al., 1989). mGluR1(693)5a was fused at Ile693 in the second intracellular loop of human mGluR1 and the corresponding amino acid residue of mGluR5a, Cys681. mGluR5a(680)1a was fused at Ile680 in the second intracellular loop of human mGluR5 and the corresponding amino acid residue of human mGluR1a, Cys694. All point mutations were constructed using a QuickChange site-directed mutagenesis kit (Stratagene, La Jolla, CA) according to the manufacturer’s instructions. For construction of truncated mGluR1a (∆N-mGluR1a), the N-terminal extracellular domain of human mGluR1a (amino acid positions 1–580) was replaced with a 20-amino acid residue sequence (MNGTEGPNFYVPFSNKTGVV) corresponding to the N terminus of bovine rhodopsin according to the method described by Zhao et al. (1999). The sequence of each mutation was confirmed by automated cycle sequencing (Applied Biosystems, Foster City, CA).

CHO K1 cells were transfected with cDNA encoding chimeras, point mutants, wild-type human mGluR1a, or human mGluR5a cloned into pcDNA3 using Lipofectamine reagent according to the manufacturer’s protocol and cultured overnight. The cells were seeded at 5 × 10⁵ cells/well in a 96-well black well/clear bottom plate and cultured overnight. Intracellular Ca²⁺ mobilization was measured using a FLIPR as described above. In the antagonist assay for mGluR1a, mGluR5, and all mutants except for the truncated mutant, final concentrations of l-glutamate were 30, 10, and 30 μM, respectively. ∆N-mGluR1a cloned into pcDNA3 was used in the IP assay as described above.

(S)-3,5-DHPG-Induced Face-Washing Behavior in Mice. Male CD1 (ICR) mice (6-weeks-old; Japan SLC, Shizuoka, Japan) were housed in a controlled animal room (room temperature: 23 ± 2°C; humidity, 55 ± 15%) on a 12-h light/dark cycle (lights on at 7:00 AM to 7:00 PM). (S)-3,5-DHPG (10 nmol) was dissolved in 0.9% NaCl, and 10 μl was intracerebroventricularly administered using a Hamilton syringe. FTIDC at doses ranging from 1 to 30 mg/kg were given i.p. or p.o. 30 min before (S)-3,5-DHPG administration. Face washing was observed from 5 to 10 min after (S)-3,5-DHPG injection. This in vivo experiment was approved by the Banyu Institutional Animal Care and Use Committee, based on adherence to the Japanese Pharmacological Society Guidance for Animal Use.

Locomotor Activity in Mice. Mice were placed into the plastic cages (21 cm (length) × 32 cm (width) × 13 cm (height)) immediately after administration of vehicle or FTIDC. Locomotor activity was measured for 60 min using an infrared motion detector system (DAS System-24A; Neuroscience, Tokyo, Japan).

### Data Analyses and Statistics

Data analyses were performed using Prism version 4.00 from GraphPad Software Inc. (San Diego, CA). Concentration-response curves for Ca²⁺ mobilization, IP production, and [³⁵S]GTPγS binding were fitted using nonlinear regression analysis. Competition binding experiments were analyzed using nonlinear regression analysis. IC₅₀ values were calculated using the Cheng-Prusoff equation: $K_i = IC_{50}/[1 + (C/K_d)]$, where $C$ is the concentration of radioligand and $K_d$ is the dissociation constant of the radioligand (Cheng and Prusoff, 1973). Student’s t test was used to analyze the mGluR1a constitutive activity data. One-way analysis of variance, followed by Dunnett’s test for multiple comparisons, was used to analyze the inverse agonist activity of FTIDC and the in vivo study. A probability level of <0.05 was considered statistically significant.

### Results

**Activity of FTIDC on Recombinant Group I mGluRs and Comparison with Other mGluR1 Antagonists.** The profiles of representative compounds identified during random screening of a chemical library, and following chemical deriveritization, are summarized in Table 1. Compound 1 was identified from our chemical library using CHO cells expressing human mGluR1α and FLIPR. Compound 2 and FTIDC were developed during successive modifications. Among these compounds, FTIDC exhibited potent antagonistic activity and good selectivity with appropriate lipophilicity. Subsequently, we characterized the in vitro and in vivo phar-

### Table 1

<table>
<thead>
<tr>
<th>Compound</th>
<th>mGluR1a (IC₅₀)</th>
<th>mGluR5 (IC₅₀)</th>
<th>Log D₇.₄</th>
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<tr>
<td>Compound 1</td>
<td>9.8 ± 0.88</td>
<td>820 ± 170</td>
<td>&gt;4.0</td>
</tr>
<tr>
<td>Compound 2</td>
<td>7.0 ± 1.2</td>
<td>170 ± 17</td>
<td>2.9</td>
</tr>
<tr>
<td>FTIDC</td>
<td>5.8 ± 0.49</td>
<td>6200 ± 520</td>
<td>2.1</td>
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macological profiles of FTIDC, the most potent and selective of the compounds screened.

The antagonistic activity of FTIDC was evaluated using cloned human mGluR1α, human mGluR1b, and rat and mouse mGluR1a. In CHO cells expressing human mGluR1α and human mGluR1b, FTIDC inhibited L-glutamate-induced increases in intracellular Ca\(^{2+}\) concentrations, with IC\(_{50}\) values of 5.8 ± 0.49 nM (n = 36) and 7.7 ± 1.4 nM (n = 7), respectively (Fig. 1A). FTIDC also inhibited L-glutamate-induced increases in intracellular Ca\(^{2+}\) concentrations in CHO cells expressing rat mGluR1α and mouse mGluR1α with similar potencies (Fig. 1A; Table 2). The IC\(_{50}\) values of FTIDC against human mGluR5 and rat mGluR5 were 6200 ± 520 nM (n = 36) and 9900 ± 40 nM (n = 5), respectively, an approximately 1000-fold weaker activity than those against mGluR1 (Fig. 1A). FTIDC exhibited no agonistic activity toward any of these group I mGluR subtypes, at least up to 10 μM (data not shown). The antagonistic activities of other mGluR1 antagonists (LY456066, R214127, YM-298198, and BAY36-7620) toward human mGluR1α were compared with the potency of FTIDC in the same assay system. Although these mGluR1 antagonists inhibited L-glutamate-induced increases in intracellular Ca\(^{2+}\) concentrations in CHO cells expressing human mGluR5, and rat mGluR5 in the presence of L-glutamate, and they are the means ± S.E.M. from more than three individual experiments performed in duplicate.

To analyze the mode of action of FTIDC, the effects of FTIDC on L-glutamate-induced increases in intracellular Ca\(^{2+}\) mobilization in CHO cells expressing human mGluR1α. Results are expressed as a percentage of the response to L-glutamate and are the means ± S.E.M. from more than three individual experiments performed in duplicate. Relative fluorescence units (RFU) in CHO cells expressing human mGluR1α, human mGluR1b, rat mGluR1α, mouse mGluR1α, human mGluR5, and rat mGluR5 in the presence of L-glutamate were 20,000 ± 840, 20,000 ± 2200, 17,000 ± 810, 12,000 ± 990, 15,000 ± 1100, and 8300 ± 2300, respectively.

**Fig. 1.** Antagonistic activities of FTIDC toward group I mGluRs. A, effect of FTIDC on L-glutamate-induced Ca\(^{2+}\) mobilization in CHO cells expressing human mGluR1α (closed squares), human mGluR1b (open squares), rat mGluR1α (closed triangles), mouse mGluR1α (open triangles), human mGluR5 (closed inverted triangles), and rat mGluR5 (open inverted triangles). B, comparison of the potency of FTIDC toward human mGluR1α with known representative mGluR1 antagonists. Results are expressed as a percentage of the response to L-glutamate and are the means ± S.E.M. from more than three individual experiments performed in duplicate. Relative fluorescence units (RFU) in CHO cells expressing human mGluR1α, human mGluR1b, rat mGluR1α, mouse mGluR1α, human mGluR5, and rat mGluR5 in the presence of L-glutamate were 20,000 ± 840, 20,000 ± 2200, 17,000 ± 810, 12,000 ± 990, 15,000 ± 1100, and 8300 ± 2300, respectively.

**Fig. 2.** Effect of FTIDC on concentration-response curves for L-glutamate-induced increases in intracellular Ca\(^{2+}\) mobilization. A, concentration-response curves for L-glutamate in the absence or presence of 3, 10, and 30 nM FTIDC in CHO cells expressing human mGluR1α. B, concentration-response curves for L-glutamate in the absence or presence of 0.1, 1, and 3 mM LY367385, a competitive antagonist in CHO cells expressing human mGluR1α. Results are expressed as a percentage of the response to 1 mM L-glutamate, and they are the means ± S.E.M. from three individual experiments performed in duplicate. RFU in CHO cells expressing human mGluR1α in the presence of 1 mM L-glutamate were 25,000 ± 3500.

**TABLE 2** Potencies (IC\(_{50}\) values) of FTIDC, YM-298198, and BAY36-7620 in inhibiting L-glutamate-induced Ca\(^{2+}\) mobilization in human, rat, and mouse mGluR1α-expressing CHO cells. IC\(_{50}\) values (nanomolar) are expressed as means ± S.E.M. (n) from more than three individual experiments performed in duplicate.

<table>
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<tr>
<th></th>
<th>FTIDC</th>
<th>YM-298198</th>
<th>BAY36-7620</th>
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<tr>
<td>Human</td>
<td>5.8 ± 0.49 (36)</td>
<td>110 ± 31 (9)</td>
<td>3300 ± 500 (3)</td>
</tr>
<tr>
<td>Rat</td>
<td>5.8 ± 0.85 (9)</td>
<td>19 ± 1.4 (10)</td>
<td>180 ± 37 (3)</td>
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<tr>
<td>Mouse</td>
<td>3.1 ± 0.27 (14)</td>
<td>20 ± 7.6 (5)</td>
<td>230 ± 28 (3)</td>
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characterization of a novel mGluR1 antagonist, FTIDC

Fig. 3. Effect of FTIDC on agonist concentration-response curves for [35S]GTPyS binding to membranes from CHO cells expressing group II and group III mGluRs. FTIDC at 10 μM was tested to investigate its effects on agonist concentration-response curves in [35S]GTPyS binding to membranes expressing mGluR2 (A), mGluR4 (B), mGluR6 (C), mGluR7 (D), and mGluR8 (E). Data are expressed as a percentage of basal [35S]GTPyS binding in the presence of 1% DMSO. Results are the means ± S.E.M. from three individual experiments performed in duplicate. The EC50 values of agonist (L-glutamate or L-AP4) in the absence of 10 μM FTIDC were 7.3 ± 1.5, 15 ± 5.9, 25 ± 5.7, 580 ± 54, and 0.94 ± 0.031 μM toward mGluR2, mGluR4, mGluR6, mGluR7, and mGluR8, respectively. The EC50 values of agonist in the presence of 10 μM FTIDC were 4.5 ± 0.57, 10 ± 1.6, 31 ± 8.2, 770 ± 46, and 0.97 ± 0.028 μM toward mGluR2, mGluR4, mGluR6, mGluR7, and mGluR8, respectively. The specific bindings of [35S]GTPyS in the presence of 1% DMSO to membranes expressing mGluR2, mGluR4, mGluR6, mGluR7, and mGluR8 were 1000 ± 100, 3000 ± 240, 3200 ± 250, 6300 ± 300, and 3300 ± 70 cpm, respectively.

curves for L-glutamate-induced increases in intracellular Ca2+ concentrations were generated in the absence or presence of FTIDC. The maximal response of L-glutamate was reduced in the presence of higher concentrations of FTIDC (Fig. 2A). In contrast, no reduction in the maximal response of L-glutamate was observed in the presence of a competitive, orthosteric mGluR1 antagonist, LY367385 (Fig. 2B).

Selectivity of FTIDC toward Other mGluR Subtypes. The selectivity of FTIDC was tested against group II and group III mGluR subtypes using the [35S]GTPyS binding assay. FTIDC at 10 μM did not exhibit any agonistic or antagonistic activity against mGluR2, mGluR4, mGluR6, mGluR7, and mGluR8. In contrast, LY341495 inhibited L-glutamate and L-AP4-induced increases in [35S]GTPyS binding to membranes from CHO cells expressing mGluR2, mGluR4, mGluR6, mGluR7, and mGluR8 (Supplemental Fig. 1). Furthermore, FTIDC was investigated to see whether it exhibited potentiated activity toward agonist-induced responses in other mGluR subtypes (mGluR2, mGluR4, mGluR6, mGluR7, and mGluR8). No significant leftward shift was observed in the agonist concentration-response curves of any of the mGluR subtypes tested in the presence of 10 μM FTIDC (Fig. 3). Furthermore, the selectivity of 10 μM FTIDC was tested against 77 target molecules such as an enzyme, neurotransmitter receptors, transporters, and ion channels, including ionotropic glutamate receptors, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, NMDA, and kainate (MDS Pharma, Bothell, WA). The IC50 values of FTIDC were higher than 10 μM against all 77 targets (data not shown).

Site of Action of FTIDC in mGluR1. [3H]-Quisqualate was used to test whether FTIDC bound to the mGluR1 L-glutamate binding site. LY367385 was also used to displace [3H]-quisqualate binding to membranes from CHO cells expressing human mGluR1α, whereas FTIDC did not (Fig. 4). Chimeric and point mutations of mGluR1 were constructed to identify the regions and amino acid residues involved in FTIDC-mediated antagonism. We initially constructed a set of chimeric mGluR1(693)5a and mGluR5(680)1a proteins fused between the third transmembrane (TM3) domain and the fourth TM domain (TM4), namely, at the second intracellular loop (Fig. 5A). L-Glutamate dose-dependently increased intracellular Ca2+ concentrations in CHO cells expressing mGluR1(693)5a and mGluR5(680)1a (data not shown). FTIDC inhibited L-glutamate-induced increases in intracellular Ca2+ concentrations in CHO cells expressing wild-type mGluR1 and mGluR5(680)1a, with IC50 values of 10 ± 0.82 (n = 9) and 9.6 ± 2.1 nM (n = 5), respectively (Fig. 5C). In contrast, IC50 values of FTIDC were shifted to 1100 ± 130 (n = 5) and 9000 ± 980 nM (n = 4) for L-glutamate-induced increases in intracellular Ca2+ concentrations in CHO cells expressing chimeric mGluR1(693)5a and wild-type mGluR5, respectively. These results indicate that the TM4 to TM7 domains of mGluR1 are important for interacting with FTIDC. Trp798, Phe801, and Tyr805 in the TM6 domain and Thr815 in the TM7 domain have been reported to be important amino acid residues for the noncompetitive mGluR1 antagonists 7-(hydroxyimino)cyclopropa[b]chromen-1a-carboxylate ethyl ester (Litschig et al., 1999) and/or EM-TBPC (Malherbe et al., 2003). Thus, point mutations of mGluR1, mGluR1(W798A), mGluR1(F801A), mGluR1(Y805A), and mGluR1(T815M) were constructed to investigate the involvement of these amino acid residues in FTIDC-mediated
antagonism (Fig. 5B). The antagonistic activities of FTIDC were significantly affected toward mGluR1a(F801A) and mGluR1a(T815M) but not toward mGluR1a(W798A) and mGluR1a(Y805A) (Fig. 5D). The IC\textsubscript{50} values of FTIDC toward mGluR1a(F801A) and mGluR1a(T815M) were 900 ± 39 nM (n = 3) and 720 ± 150 nM (n = 4), respectively. In contrast, the IC\textsubscript{50} values of FTIDC toward mGluR1a(W798A) and mGluR1a(Y805A) were 8.0 ± 0.95 nM (n = 3) and 14 ± 2.5 nM (n = 3), respectively.

**Inverse Agonist Activity of FTIDC.** FTIDC was tested to see whether it had inverse agonist activity against mGluR1. Higher basal IP production was observed in HEK293 cells transiently cotransfected with human mGluR1a and rGLAST compared with control cells transfected with rGLAST (Fig. 6A).

FTIDC dose-dependently inhibited basal IP production in HEK293 cells expressing human mGluR1a, with an IC\textsubscript{50} value of 7.0 ± 0.73 nM (n = 7; Fig. 6B). FTIDC also inhibited 100 μM L-glutamate-induced increases in IP production, with an IC\textsubscript{50} value of 5.3 ± 1.1 nM (n = 4; data not shown). To clarify the interaction of FTIDC with the TM domain of mGluR1 in regulating constitutive activity, N-terminal truncated mGluR1a (ΔN-mGluR1a) was constructed. ΔN-mGluR1a was derived from the first 20 amino acid residues of bovine rhodopsin followed by the mGluR1a sequence beginning with amino acid residue 581 (Fig. 6C). Basal IP production in HEK293 cells transiently transfected with ΔN-mGluR1a was still higher than those in control cells (Fig. 6D). Basal IP production in HEK293 cells expressing ΔN-mGluR1a was not increased by L-glutamate even at 10 mM (Fig. 6D), whereas 100 μM L-glutamate induced increases in IP production in HEK293 cells expressing wild-type human mGluR1a (Fig. 6A). FTIDC still inhibited basal IP production in HEK293 cells expressing ΔN-mGluR1a.

![Figure 5](image_url)

Fig. 5. Effect of FTIDC on L-glutamate-induced intracellular Ca\textsuperscript{2+} mobilization in CHO cells expressing mGluR1a chimeras and mGluR1a point mutations. A, schematic diagrams of chimeric receptors mGluR1(693)5a and mGluR5(680)1a, indicating the location of fusion sites between human mGluR1a and human mGluR5a. B, amino acid sequence of TM6 and part of TM7 in human mGluR1a. Underlined amino acid residues represent positions mutated in the present study. C, effect of FTIDC on L-glutamate-induced intracellular Ca\textsuperscript{2+} mobilization in CHO cells expressing wild-type mGluR1a (closed squares), mGluR1(693)5a (open squares), mGluR5(680)1a (closed triangles), and wild-type mGluR5a (open triangles). D, effect of FTIDC on L-glutamate-induced intracellular Ca\textsuperscript{2+} mobilization in CHO cells expressing wild-type mGluR1a (closed squares), mutant mGluR1a(W798A) (open squares), mGluR1a(F801A) (closed triangles), mGluR1a(T815M) (open triangles), or mGluR1a(T815M) (closed inverted triangles). Results are expressed as a percentage of the response to L-glutamate, and they are the means ± S.E.M. from more than three individual experiments performed in duplicate. RFU in CHO cells expressing mGluR1a, mGluR1(693)5a, mGluR5(680)1a, mGluR5, mGluR1a(W798A), mGluR1a(F801A), and mGluR1a(T815M) in the presence of L-glutamate were 8400 ± 1400, 5900 ± 1200, 4400 ± 1000, 6600 ± 650, 9200 ± 1200, 6200 ± 1000, 7500 ± 1000, and 4400 ± 320, respectively.

![Figure 6](image_url)

Fig. 6. Effect of FTIDC on basal IP production in HEK293 cells expressing human mGluR1a. A, basal and 100 μM L-glutamate-induced IP productions in HEK293 cells expressing human mGluR1a and basal IP production in control cells (mock). B, effect of FTIDC on basal IP production in HEK293 cells expressing human mGluR1a. C, schematic diagrams of wild-type mGluR1a and N-terminal truncated mGluR1a (ΔN-mGluR1a). ΔN-mGluR1a was derived from the first 20 amino acid residues of bovine rhodopsin followed by the mGluR1a sequence beginning with amino acid residue 581. D, IP production in the absence or presence of 10 mM L-glutamate in HEK293 cells expressing ΔN-mGluR1a and basal IP production in control cells (mock). E, effect of FTIDC on basal IP production in HEK293 cells expressing ΔN-mGluR1a. Results are the means ± S.E.M. from more than four individual experiments performed in quadruplicate. Statistical analyses were conducted with one-way analysis of variance followed by post hoc multiple comparison test (Dunnett’s test). **, P < 0.01 versus control.
mGluR1a with an IC_{50} value of 2.7 ± 0.53 nM (n = 4; Fig. 6E).

**In Vivo Activity of FTIDC in Mice.** To determine the in vivo antagonistic activity of FTIDC, the effect of FTIDC on (S)-3,5-DHPG-induced face-washing behavior was investigated in mice. An intracerebroventricular administration of 10 nmol of (S)-3,5-DHPG resulted in increased face-washing behavior. Pretreatment of FTIDC (i.p.) reduced the duration of face-washing behavior elicited by (S)-3,5-DHPG in a dose-dependent manner (Fig. 7A), and the inhibitory effect of FTIDC was statistically significant at 10 and 30 mg/kg. The effect of FTIDC on locomotor activity was evaluated in mice administered vehicle, 1, 3, 10, and 30 mg/kg (i.p.) FTIDC. There is no significant difference in locomotor activity in the FTIDC-treated groups compared with the vehicle group (Fig. 8).

To evaluate the oral activity of FTIDC, the effect on (S)-3,5-DHPG-induced face-washing behavior was investigated after p.o. administration of FTIDC. Like i.p. administration, orally administered FTIDC inhibited (S)-3,5-DHPG-induced face-washing behavior in a dose-dependent manner, with the effect at a dose of 30 mg/kg p.o. being statistically significant (Fig. 7B). The concentrations of FTIDC in plasma and brain were determined to examine its brain penetrability after oral dosing. The concentrations of FTIDC in plasma and brain were 0.21 ± 0.074 μM and 0.17 ± 0.063 nmol/g (mean ± S.E.M. from three mice) at 30 min after oral administration (30 mg/kg).

**Discussion**

We have identified a potent and selective mGluR1 antagonist from a new class of chemicals. Compound 1 (Table 1) was one of the prototype compounds identified from our chemical library by high-throughput screening. Compound 1 is a potent antagonist with moderate selectivity and high lipophilicity (log D_{7.4} = 4.0). The lipophilicity of compound 1 could be problematic because the optimal range of log D_{7.4} values in orally active CNS drugs is generally thought to be between 1 and 3 (Comer, 2003). In an attempt to reduce the lipophilicity of the lead compound, compound 2 (Table 1) was identified as a potent, less lipophilic mGluR1 antagonist. However, compound 2 exhibited less selectivity than compound 1. In the course of successive modifications, FTIDC was obtained as a potent and selective mGluR1 antagonist with appropriate lipophilicity. FTIDC is a compound that has a unique tetracydropyridine structure. Therefore, its chemical structure is different from known competitive and non-competitive mGluR1 antagonists (Layton, 2005; Supplemental Fig. 2).

FTIDC equally inhibited l-glutamate-induced intracellular Ca^{2+} mobilization in CHO cells expressing human, rat, or mouse mGluR1a. In the present study, YM-298198 and BAY36-7620 were less potent toward human mGluR1a than toward rat or mouse mGluR1a, although the potencies of these compounds toward human mGluR1 were not described in the original reports (Carroll et al., 2001; Kohara et al., 2005). Therefore, unlike these known antagonists, FTIDC exhibits potent antagonistic activity toward both rodent and human mGluR1.

As well as mGluR1a, mGluR1b is one of major splice variants of mGluR1 in mammalian brain (Soloviev et al., 1999), and it lacks the long carboxyl-terminal intracellular domain characteristic of mGluR1a (Conn and Pin, 1997). FTIDC inhibited the l-glutamate-induced activation of mGluR1b and mGluR1a with similar potency. This result suggests that antagonistic activity of FTIDC is not affected by the absence of the carboxyl-terminal intracellular domain.

FTIDC is approximately 1000-fold more selective toward mGluR1a than toward mGluR5. FTIDC also showed neither agonist nor antagonist activity toward the other mGluR subtypes studied: mGluR2, mGluR4, mGluR6, mGluR7, and mGluR8. These results indicate that FTIDC is a highly selective mGluR1 antagonist among mGluR subtypes. Noncompetitive mGluR antagonists such as N-phenyl-7-(hydroxymethyl)cyclopropa[b]chromen-1a-carboxamide, SIB-1893, and MPEP act as positive allosteric modulators toward mGluR4 (Maj et al., 2003; Mathiesien et al., 2003). Thus, we evaluated whether FTIDC could act as a positive allosteric modulator.
FIDC decreased the maximal L-glutamate-induced Ca\(^{2+}\) mobilization in CHO cells expressing human mGluR1a in a noncompetitive manner, unlike the competitive amino acid agonist LY367385. Furthermore, FIDC did not displace \(^{3}\)H]-quisqualate binding to membranes prepared from CHO cells expressing mGluR1a. Taken together, these results indicate that FIDC is one of the most highly selective mGluR1 antagonists among known antagonists.

The lack of displacement of \(^{3}\)H]-quisqualate prompted us to investigate the site of action of FIDC. The TM domains of the mGluR family are known to be important for the action of known allosteric mGluR ligands (Litschig et al., 1999; Pagano et al., 2000; Maj et al., 2003; Malherbe et al., 2003; Schaffhauser et al., 2003). The activity of FIDC toward chimeric mGlur5(680)1a was similar to that toward wild-type mGlur1a. The activity of FIDC toward chimeric mGlur1(693)5a was much less than toward wild-type mGlur1a and mGlur5(680)1a and similar to that toward wild-type mGlur5a. These results suggest that FIDC can interact with the region spanning TM4 to TM7 of mGluR1, consistent with known allosteric mGluR1 antagonists. We cannot exclude the involvement of amino acid residues upstream of the second intracellular loop domain, because the activity of FIDC toward mGlur1(693)5a was slightly higher than toward wild-type mGlur5a. However, the present study suggests that the region spanning TM4 to TM7 is more important than other regions for FIDC-mediated antagonism toward mGlur1.

A noncompetitive mGlur1 antagonist, 7-(hydroxyimino)cyclo-propa[b]chromen-1a-carboxylate ethyl ester, interacts with Thr815 in TM7 of mGlur1 (Litschig et al., 1999). The mutation of Phe801, Tyr805, or Thr815 in TM6 or TM7 of mGlur1 caused complete loss of ability to bind a potent mGlur1 antagonist, \(^{3}\)H]-EM-TBPC (Malherbe et al., 2003). In addition, the mutation of Trp798 in TM6 of mGlur1 increased the binding affinity of \(^{3}\)H]-EM-TBPC about 10-fold. Therefore, the effects of FIDC on these mGlur1 mutants were evaluated. The concentration-response curve of FIDC was clearly shifted toward the right in CHO cells expressing mGlur1a(F801A) or mGlur1a(T815M) compared with CHO cells expressing wild-type human mGlur1a. In contrast, there is no significant shift in the concentration-response curve in CHO cells expressing mGlur1a(W798A) or mGlur1a(Y805A). These results suggest that Phe801 in TM6 and Thr815 in TM7 are involved in FIDC-mediated antagonism toward mGlur1. In contrast, unlike EM-TBPC, Trp798 and Tyr805 in TM6 had less effect on FIDC-mediated antagonism. These results suggest that FIDC at least partially interacts with the same amino acid residues as interact with known noncompetitive mGlur1 antagonists, but it may not use the identical binding site. To more precisely reveal the FIDC site of action, systematic mutational analyses, in combination with a three-dimensional model of the mGlur1 TM domain, will be necessary.

Known allosteric mGlur1 antagonists such as BAY36-7620, JNJ16259685, and YM-298198 show inverse agonist activity toward mGlur1a (Carroll et al., 2001; Lavreyansen et al., 2004; Kohara et al., 2005). Like these compounds, FIDC inhibited basal IP production in HEK293 cells expressing human mGlur1a. An allosteric mGlur5 antagonist, MPEP, displayed inverse agonist activity toward mGlur5 (Pagano et al., 2000), and it inhibited basal IP production in cells expressing truncated mGlur5 lacking the N-terminal large extracellular domain (Goudet et al., 2004). These results suggest that MPEP could exert its inverse agonist activity independent of this N-terminal extracellular domain. Basal IP production in HEK293 cells expressing \(\Delta N\)mGlur1a was higher than that in control cells. Even at 10 mM, L-glutamate did not increase IP production in cells expressing \(\Delta N\)mGlur1a, consistent with the absence of the L-glutamate binding site. FIDC inhibited basal IP production in HEK293 cells expressing \(\Delta N\)mGlur1a. These results further support the conclusion that FIDC acts as an inverse agonist toward mGlur1a and indicate that, like MPEP, FIDC does not require the N-terminal large extracellular domain of mGlur1 to exert its inverse agonist activity. This is the first demonstration that constitutive activity of mGlur1 can be regulated by only the TM domain, independently of the mGlur1 N-terminal large extracellular domain.

We assessed the in vivo activity of FIDC toward (S)-3,5-DHPG-induced face-washing behavior in mice. (S)-3,5-DHPG is a specific group I mGlur agonist with similar affinities for mGlur1 and mGlur5 (Pin et al., 1999). An intracerebroventricular administration of (S)-3,5-DHPG elicited behavioral changes in mice, including facial grooming (Barton and Shannon, 2005). We also observed similar behavior following intracerebroventricular administration of (S)-3,5-DHPG. The behavioral changes produced by (S)-3,5-DHPG were antagonized by a mGlur1 antagonist, LY456236 (i.p.), but not by a mGlur5 antagonist, MPEP (Barton and Shannon, 2005). These results indicate that face-washing behavior produced by (S)-3,5-DHPG is due to activation of mGlur1 but not mGlur5. FIDC inhibited (S)-3,5-DHPG-induced face-washing behavior in a dose-dependent manner. In addition, YM-298198 also inhibited the behavioral changes produced by (S)-3,5-DHPG at 10 and 30 mg/kg (s.c.) (Supplemental Fig. 3). Inhibition of (S)-3,5-DHPG-induced face-washing behavior by structurally diverse mGlur1 antagonists LY456236, FIDC, and YM-298198 supported that the behavioral changes are mediated by mGlur1 activation. However, non-NMDA receptor antagonists inhibited (S)-3,5-DHPG-induced face-washing behavior at doses producing motor impairment (Barton and Shannon, 2005). In the present study, FIDC did not significantly decrease locomotor activity at doses suppressing (S)-(S)-3,5-DHPG-induced face-washing behavior. These results indicated that the inhibitory action of FIDC on (S)-(S)-3,5-DHPG-induced face-washing behavior could be due to inhibition of mGlur1 but not to motor impairment. Finally, receptor occupancy studies with mGlur1 selective radioligands will be necessary to demonstrate the further direct involvement of mGlur1 in (S)-(S)-3,5-DHPG-induced face-washing behavior. Oral administration of FIDC also inhibited the face-washing behavior induced by (S)-(S)-3,5-DHPG.
Characterization of a Novel mGluR1 Antagonist, FTIDC

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