Unique Antipsychotic Activities of the Selective Metabotropic Glutamate Receptor 1 Allosteric Antagonist 2-Cyclopropyl-5-[1-(2-fluoro-3-pyridinyl)-5-methyl-1H-1,2,3-triazol-4-yl]-2,3-dihydro-1H-isoindol-1-one

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ABSTRACT

A newly discovered metabotropic glutamate receptor (mGluR) 1 allosteric antagonist, 2-cyclopropyl-5-[1-(2-fluoro-3-pyridinyl)-5-methyl-1H-1,2,3-triazol-4-yl]-2,3-dihydro-1H-isoindol-1-one (CFMTI), was tested both in vitro and in vivo for its pharmacological effects. CFMTI demonstrated potent and selective antagonistic activity on mGluR1 in vitro and in vivo after oral administration. CFMTI inhibited L-glutamate-induced intracellular Ca2+ mobilization in Chinese hamster ovary cells expressing human and rat mGluR1, with IC50 values of 2.6 and 2.3 nM, respectively. The selectivity of CFMTI to mGluR1 over mGluR5 was 2000-fold, and CFMTI at 10 μM showed no agonistic or antagonistic activities toward other mGluR subtypes and other receptors. It antagonized face-washing behavior in mice induced by (S)-3,5-dihydroxyphenylglycine at a dose range of 3 to 30 mg/kg, for which receptor occupancy was 73 to 94%. As with the classical neuroleptic haloperidol and an atypical antipsychotic, clozapine, orally administered CFMTI reduced methamphetamine-induced hyperlocomotion and disruption of prepulse inhibition (PPI) at the same dose range as required to antagonize the face-washing behavior. CFMTI and clozapine improved ketamine-induced hyperlocomotion, PPI disruption and (5S,10R)-(+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine maleate (MK-801)-induced social withdrawal without any cataleptogenic activities, whereas haloperidol only improved ketamine-induced hyperlocomotion. CFMTI, unlike clozapine, caused neither hypolocomotion nor motor incoordination at therapeutic doses. In c-fos expression studies, CFMTI and clozapine increased the number of fos-positive neurons in the nucleus accumbens and medial prefrontal cortex but not in the dorsolateral striatum. These results suggest that the antipsychotic activities of mGluR1 antagonists are more similar to those of atypical antipsychotics than those of typical antipsychotics.

1-L-Glutamate is a major excitatory neurotransmitter in the mammalian central nervous system (CNS) and acts on both ligand-gated ion channels (ionotropic glutamate receptors; iGluRs) and G protein-coupled metabotropic glutamate receptors (mGluRs). The mGluR family consists of eight recep-

ABBREVIATIONS: CNS, central nervous system; iGluR, ionotropic glutamate receptor; mGluR, metabotropic glutamate receptor; FTIDC, 4-[1-(2-fluoropyridin-3-yl)-5-methyl-1H-1,2,3-triazol-4-yl]-N-isopropyl-N-methyl-3,6-dihydro(dipyrindine-1(2H)-carboxamide; MAP, methamphetamine; CFMTI, 2-cyclopropyl-5-[1-(2-fluoro-3-pyridinyl)-5-methyl-1H-1,2,3-triazol-4-yl]-2,3-dihydro-1H-isoindol-1-one; NMDA, N-methyl-D-aspartate; PPI, prepulse inhibition; GTPγS, guanosine 5′-O-(3-thiotriphosphate; DHPG, (S)-3,5-dihydroxyphenylglycine; MK-801, (5S,10R)-(+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine maleate; CHO, Chinese hamster ovary; hM, human metabolotropic; MOPS, 3-(N-morpholino)propanesulfonic acid; FTIDC, 4-[1-(2-fluoropyridin-3-yl)-5-methyl-1H-1,2,3-triazol-4-yl]-N-isopropyl-N-methyl-3,6-dihydro(dipyrindine-1(2H)-carboxamide; BSA, bovine serum albumin; PBS, phosphate-buffered saline; TPBS, 0.3% Triton X-100 in phosphate-buffered saline; NAC, nucleus accumbens; dSTr, dorsolateral part of the rostral striatum; mPFC, medial prefrontal cortex; R214127, 1-(3,4-dihydro-2H-pyran-2-yl)-5-methyl-10-(1H-tetrazol-5-yl)-2-phenyl-1-ethanone; JNJ16259685, (3,4-dihydro-2H-pyran-2-yl)-5-methyl-10-(1H-tetrazol-5-yl)-2-phenyl-1-ethanone; EMQCMC, (3-ethyl-2-methyl-quinolin-6-yl)(4-methoxy-cyclohexyl)methanone; 6M, methanesulfonate; BAY 36-7620, (3S,6aS,6a-ethanol-2-ylmethyl-5-methyliden-hexahydro(cyclopenten)1(4H)-one-10-imine; PCP, phencyclidine; LY354740, (S)-2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylate monohydrate; LY367385, (+)-2-methyl-4-carboxyphenylglycine.
tor subtypes that are divided into group I (mGluR1 and mGluR5), group II (mGluR2 and mGluR3), and group III (mGluR4, mGluR6, mGluR7, and mGluR8). The receptors are categorized based on their sequence homology, pharmacological profiles, and signal transduction pathways (De Blasi et al., 2001; Spooren et al., 2003). In neurons, iGluRs and group I mGluRs are mainly localized in somatodendritic domains and postsynaptically regulate neuronal excitability, whereas group II and III mGluRs are predominantly localized in axonal domains and terminals to presynaptically regulate neuronal excitability, as described in Blasi et al., 2001; Spooren et al., 2003. In neurons, iGluRs are categorized based on their sequence homology, pharmacological properties, and signal transduction pathways, as described in Blasi et al., 2001; Spooren et al., 2003. In neurons, iGluRs are categorized based on their sequence homology, pharmacological properties, and signal transduction pathways, as described in Blasi et al., 2001; Spooren et al., 2003.

Recently, we discovered a potent and selective mGluR1 allosteric antagonist, FTIDC, which inhibits the psycho-stimulant, methamphetamine (MAP)-induced behavioral alterations such as hyperlocomotion and disruption of prepulse inhibition (PPI) at intraperitoneal doses of 10 and 30 mg/kg (Suzuki et al., 2007; Satow et al., 2008). These results suggest that blockade of mGluR1 mimics some effects of antipsychotics. To explore potential antipsychotic effects due to blockade of mGluR1, we developed a novel, orally active, potent, and selective mGluR1 allosteric antagonist, 2-cyclopropyl-5-[1-(2-fluoro-3-pyridinyl)-5-methyl-1H-1,2,3-triazol-4-yl]-2,3-dihydro-1H-isodol-1-one (CFMTI), by chemical modification of FTIDC.

The aim of this study was to expand our original finding that blockade of mGluR1 suppresses dopamine-related psychotic-like behavior. We examined CFMTI in various animal models for schizophrenia, including NMDA antagonist-induced behavioral alterations and compared the effects of an mGluR1 antagonist with those of known typical and atypical antipsychotic agents, such as haloperidol and clozapine. Here, we report that CFMTI improves NMDA antagonist-induced hyperlocomotion, deficits in PPI and social interaction, as well as MAP-induced hyperlocomotion and PPI disruption, at doses where CFMTI occupied most mGluR1 receptors. In addition, c-fos expression in various brain regions was studied after administration of CFMTI to rats. The neurochemical profiles of CFMTI were similar to those of an atypical antipsychotic, clozapine, but not to those of a typical antipsychotic, haloperidol. Thus, the antipsychotic-like effects of CFMTI were similar to those of atypical antipsychotic agents but not of typical agents.

**Materials and Methods**

**Animals.** All experiments were performed using adult male CD1 (ICR) mice (Japan SLC, Shizuoka, Japan) and Sprague-Dawley rats (Charles River Laboratories Japan, Kanagawa, Japan). Animals were housed in an air-conditioned room with a 12/12-h light/dark cycle (lights off at 7:00 PM) and allowed ad libitum access to food (CE-2; Clea Japan, Inc., Tokyo, Japan) and tap water. At least 6 days were allowed for acclimatization to the facility before starting experiments. All experiments were approved by our Institutional Animal Care and Use Committee.

**Compounds and Administration Procedures.** The structure of CFMTI is shown in Fig. 1. CFMTI, FTIDC, and (3H)FTIDC were synthesized in-house and used in all experiments as the free base. CFMTI was suspended in 0.5% methylcellulose for oral administration. The behavioral studies were conducted between 30 and 60 min after administration, with the specific time noted in each behavioral assay. The dosing volume was 10 and 1 ml/kg for mice and rats, respectively. 1-Glutamate was purchased from Sigma-Aldrich (St. Louis, MO). (3H)Quisqualate (31–33 Ci/mmol) and [35S]GTPγS (1000 Ci/mmol) were purchased from GE Healthcare (Chalfont St. Giles, Buckinghamshire, UK). Dialyzed fetal bovine serum, culture media, and other reagents used for cell culture were purchased from Invitrogen (Carlsbad, CA). All other reagents used in vitro assays were of molecular or analytical grade where appropriate. (S)-3,5-Dihydroxyphenylglycine (DHPG; Tocris Bioscience, Ellisville, MO) was dissolved in physiological saline for intracerebroventricular administration. MAP (Dainippon Sumitomo Pharma, Osaka, Japan), ketamine (Daichi Sankyo Propharma Co., Ltd., Tokyo, Japan), and MK-801 (Sigma-Aldrich) were dissolved in physiological saline for subcutaneous administration. The following drugs were used as positive controls to demonstrate the sensitivity of the methods in appropriate animal models. Haloperidol (Serenece; Dainippon Sumitomo Pharma) was diluted with distilled water for subcutaneous administration. Clozapine (Tocris Bioscience) was dissolved in HCl and then diluted with physiological saline for subcutaneous injection. For the social interaction tests, haloperidol was diluted with distilled water, and clozapine was suspended in 0.5% methylcellulose for oral administration.

**Stable Cell Lines.** CHO-dihydrofolute reductase− cells stably expressing human mGluR1a (CHO-hmGluR1a) and human mGluR2 (CHO-hmGluR2) were obtained as described previously by Ohashi et al. (2002) and Suzuki et al. (2007), respectively. CHO-dihydrofolute reductase− cells stably expressing rat mGluR1a (CHO-rmGluR1a) were kindly donated by Dr. S. Nakanishi (Kyoto University, Kyoto, Japan). CHO cell lines stably expressing human mGluR4 (CHO-hmGluR4), human mGluR5 (CHO-hmGluR5), human mGluR6 (CHO-hmGluR6), and human mGluR7 (CHO-hmGluR7) were obtained as described previously by O’Brien et al. (2004). The CHO cell line stably expressing human mGluR8 (CHO-hmGluR8) was obtained as described previously by Suzuki et al. (2007).

**Membrane Preparation.** Membranes were prepared according to the method described by Suzuki et al. (2007). In brief, 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 homogenized using a Polytron homogenizer (Kinematica, Littau-Lucerne, 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, 1 mM EDTA, 10 mM EGTA, 10 mM MgCl2, 10 mM MnCl2, 0.1% CHAPS, pH 7.4) containing 600 mM NaCl, 250 mM sucrose, and 1 mM DTT.
mM HEPES, pH 7.4, and 0.1 mM EDTA) supplemented with protease inhibitor cocktail (Complete EDTA-free; Roche Diagnostics, Mannheim, Germany) and centrifuged at 100,000g for 60 min at 4°C. The pellet was resuspended in buffer B and stored in aliquots at −80°C.

Intracellular Ca²⁺ Mobilization. Intracellular Ca²⁺ mobilization assays were conducted according to previously described methods (Suzuki et al., 2007). CHO-hmGluR1a, CHO-rmGluR1a, and CHO-hmGluR5 were seeded in a 96-well plate and cultured overnight. The cells were then incubated with 4 μM Fluo-3 (mGluR1) or Fluo-4 (mGluR5) for 1 h, and then extracellular dye was removed by washing the cells. Ca²⁺ flux was measured using a fluorometric imaging plate reader (Molecular Devices, Sunnyvale, CA). Test compounds were applied 5 min before the application of L-glutamate. The final concentrations of L-glutamate for CHO-hmGluR1a, CHO-rmGluR1a, and CHO-hmGluR5 were 10, 30, and 10 μM, respectively.

³²P[S]GTPγS Binding. [³²P]GTPγS binding studies were carried out according to the method described by Suzuki et al. (2007). In brief, membranes prepared from CHO cells expressing mGluR2 were incubated with test compounds and 400 pM [³²P]GTPγS in 20 mM HEPES, 100 mM NaCl, 1 mM MgCl₂, 1 mM EDTA, and 5 μM GDP, pH 7.4, and 1.5 mg of wheat germ agglutinin-coated SPA beads (GE Healthcare) at 25°C for 2 h in the absence or presence of 100 μM L-glutamate. Membrane-bound radioactivity was detected by scintillation proximity using a TopCount microplate scintillation counter (PerkinElmer Life and Analytical Sciences, Boston, MA). For the mGluR4 assay, incubation time and concentration of L-glutamate were 1 and 10 μM, respectively. For the mGluR6 assay, incubation time and concentration of L-glutamate were 1.5 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-2-amino-4-phosphonobutyric acid. The effect of CFMTI on agonist concentration-response curves for mGluR2, mGluR4, mGluR6, mGluR7, and mGluR8 was tested to evaluate positive allosteric modulator activity of CFMTI. Dose-response curves of L-glutamate for mGluR2, mGluR4, mGluR6, and mGluR8, or L-2-amino-4-phosphonobutyric acid for mGluR7 were generated and compared in the absence and presence of 10 μM CFMTI.

[³²P]GTPγS binding assay were carried out as described above.

[³²P]H]-L-Quisqualate Binding. [³²P]H]-L-Quisqualate binding studies were carried out according to the method described by Suzuki et al. (2007). In brief, membranes prepared from CHO cells expressing human mGluR1a were incubated with 50 nM [³²P]-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 GF/C filter plates and a Filtermate 196 harvester (PerkinElmer Life and Analytical Sciences). Radioactivity trapped on the filter was counted with a TopCount instrument after the addition of MicroScint 0 (PerkinElmer Life and Analytical Sciences).

Pharmacokinetic Study in Rats. Plasma concentration-time profiles of CFMTI and FTIDC were studied in conscious rats (n = 3/group). CFMTI and FTIDC were dissolved in ethanol, polyethylene glycol 400, and distilled water (1:4:5, v/v/v) for oral administration. The drugs were suspended in 0.5% methylcellulose for oral administration. CFMTI and FTIDC were dissolved in ethanol, polyethylene glycol 400, and distilled water (1:4:5, v/v/v) for intravenous administration. Approximately 100–300 mg/kg of the drug from the receptor. After incubation, the sections were washed (3 × 4 min) with ice-cold 50 mM Tris containing 1.2 mM MgCl₂ and 2 mM CaCl₂, pH 7.4, and 0.1% bovine serum albumin (BSA) for 3 min at room temperature. Incubation was restricted to 3 min to minimize dissociation of the drug from the receptor. After incubation, the sections were washed (3 × 4 min) with ice-cold 50 mM Tris containing 1.2 mM MgCl₂ and 2 mM CaCl₂, pH 7.4. The sections were then dried under a stream of air, and radioisotopic binding was evaluated using a β-imager 2000 (BioSpace, Paris, France). The levels of bound radioactivity in various brain areas were determined by counting the number of β-particles emerging from the delineated area (region of interest) using the Biorad imaging system (version 2.0). Consequently, the radioisotopic binding signal was expressed in counts per minute per square millimeter.

Total binding of [³²P]H]-FTIDC in each mouse was obtained by calculating the mean value of radioactivity from six brain sections. Non-specific binding was defined as the radioactivity in the presence of 10 μM unlabeled FTIDC and was obtained by calculating the mean value of nine brain sections from three mice treated with vehicle (three brain sections/mouse). Specific binding of [³²P]H]-FTIDC in each mouse was calculated by subtracting the nonspecific binding from the total binding. Specific binding of [³²P]H]-FTIDC in drug-treated mice was expressed as the percentage of specific binding in vehicle-treated animals. Receptor occupancy by the drug was expressed as 100% minus the percentage of specific binding in the treated animals.

Plasma samples and brain homogenates were analyzed for the determination of compound concentration by liquid chromatography/ tandem mass spectrometry. Compounds in samples were extracted with ethanol containing the internal standard. Quantitative analy-
ses were performed by the relative calibration curve method using the authentic standard and the internal standard.

Spontaneous Locomotor Activity under Nonhabituated Condition and MAP-Induced Hyperlocomotion in Mice. Locomotor activity of ICR mice (n = 6–17/group) was measured by an activity-monitoring system (NS-AS01; Neuroscience) as described above. Animals were administered test compounds, and nonhabituated spontaneous locomotor activity was measured in the test cage (22.5 × 33.8 × 14.0 cm, length × width × height) for 1 h. In the drug interaction study, tested compounds were administered 30 min before MAP was subcutaneously injected at 2 mg/kg. Immediately after MAP injection, the mouse was placed into the test cage, and locomotor activity was measured for 1 h.

Ketamine-Induced Hyperlocomotion under Habitudated Condition in Mice. Locomotor activity of ICR mice (n = 4–19/group) was measured by an activity-monitoring system (NS-AS01; Neuroscience) as described above. Because ketamine-induced hyperlocomotion in mice was observed in well habituated animals, mice were placed into the test cage (22.5 × 33.8 × 14.0 cm, length × width × height) after administration of CFMTI, haloperidol, or clozapine. Thirty minutes after drug injection, ketamine was subcutaneously injected at 10 mg/kg. Immediately after ketamine injection, locomotor activity was measured for 1 h.

Measurement of PPI in Rats. Sprague-Dawley rats were used to measure PPI in the Startle Response System (SR-Lab; San Diego Instruments, San Diego, CA). The whole apparatus was housed in a ventilated chamber (39 × 38 × 58 cm, length × width × height). Presentation of acoustic stimulii was controlled by SR-Lab software and interface that digitized, rectified, and recorded responses from the accelerometer on a personal computer. Mean startle amplitude was determined by averaging 100 1-ms readings taken at the pulse stimulus onset. On the day of PPI testing, rats (n = 9–13/group) were placed into the startle apparatus. After a period of 5 min, during which rats were exposed to background noise (60 dB), the session began with rats being exposed to different auditory stimuli. The session began with 15 of each of the following six trial types: 1) startle stimulus (120 dB; 40 ms); 2 to 4) startle stimulus preceded by 100 ms of prepulses at 3, 6, and 12 dB above background noise (60 dB) and lasting 20 ms; 5) prepulse stimulus (72 dB; 20 ms); and 6) no stimulus. These six trial types were interspersed across 15 consecutive blocks. The intertrial interval was pseudorandomly varied between 15 and 30 s, and sessions lasted approximately 35 min. Data collected in the first five blocks were discarded due to large variance in the startle responses; data from the last 10 blocks were analyzed. Haloperidol or clozapine was subcutaneously administered 15 min before the beginning of the session, and CFMTI was administered 30 min before the session. In the drug interaction studies, MAP at 3 mg/kg and ketamine at 5 mg/kg were subcutaneously injected 10 and 5 min before the session, respectively.

MK-801-Induced Deficits in Social Interaction in Rats. Social interaction tests (n = 6–15/group) were performed using a gray-colored polyvinyl chloride box (62.4 × 41.7 × 36.9 cm, length × width × height) with six divided areas. Each social interaction test between two rats was carried out during the light phase of the light/dark cycle. Rats were selected from separate housing cages to make a pair for the study. The body weights of the paired rats were matched within 20 g of variance. All rats were placed in an experimental sound-proof room, and the study was conducted 4 h after the subcutaneous administration of MK-801 at 0.1 mg/kg. Each pair of rats was diagonally placed in opposite corners of the box so that they faced away from each other. Saline was administered as the vehicle. CFMTI was orally administered 30 min before the social interaction test (3.5 h after the injection of MK-801). Haloperidol or clozapine were orally administered 60 min before the test (3 h after the injection of MK-801), because subcutaneous administration of these drugs caused sedation that interfered with social interaction. The behavior of the animals was monitored and recorded on a video recorder located outside the box over a 5-min period. All behavioral observations were conducted through a monitor on live feed or recorded. The test box was wiped clean between each trial. Social interaction between two rats was determined as the total time spent participating in social behavior such as sniffing, genital investigation, chasing and fighting each other. In addition, locomotor activity of each animal was measured as the number of lines (marked on the floor of the box) that the animal crossed during the test.

Catalepsy Assay in Rats. Catalepsy was determined by placing the front paws of the animal over a rod raised approximately 10 cm above the floor. The duration for which the animal remained in this forced position was measured with a “cut-off” of 60 s. The measurement procedure was repeated three times, and the longest duration that the raised rod was held with the front paws was used for subsequent analysis. Test compounds were administered to the rats (n = 5–11/group) 60 min before this study.

Rotarod Test in Mice. Effects of test compounds on motor coordination of ICR mice were evaluated using a Rotarod apparatus (model 7650; Ugo Basile, Comerio, Italy). Mice were trained twice on the apparatus on the day of testing. In the first training session, mice were trained to walk on the rotating rod (8 revolutions/min) for 5 min. Approximately 1 h after the first training session, mice that had walked for at least 3 min without falling off were selected for the second training session. Rotarod performance before drug administration in selected mice was given as 120 s. In the test session, the duration that mice (n = 5–32/group) walked on the rotating rod was measured 30, 60, and 120 min after administration of the test compounds.

Effects of CFMTI on Object Location Memory in Mice. We used methods of object location tests in mice as reported previously (Murai et al., 2007). In brief, the experimental arena was made of gray polyvinyl chloride open-field box (40 × 30 × 30 cm, length × width × height), which was illuminated by a fluorescent light above the box. Identical plastic columns (4 cm in height × 5 cm in diameter) were used as objects. The mice were allowed to freely explore the arena without objects for 1 h on the day before the acquisition trial. On the acquisition trial, each mouse was allowed 5 min to explore two identical objects that were placed in two of four corners of the arena. CFMTI was orally administered 30 min before the acquisition trial. The mouse was then removed from the arena and returned to its home cage. One hour after the acquisition trial, one object was moved to other vacant corner. Then, the mouse was reintroduced into the arena for 5 min, and its behavior was recorded with a video camera mounted above the apparatus. The time spent for exploring each object (TND and TD) was recorded. To analyze cognitive performance, a location index was calculated as follows: location index (%) = (TND × 100)/(TD + TND), where TND and TD are time spent for exploring the displaced and nondisplaced object, respectively.

Immunohistochemical Analysis of c-Fos Expression in Rats. CFMTI (10–30 mg/kg p.o.), haloperidol (1 mg/kg s.c.), or clozapine (30 mg/kg i.p.) was administered to rats (n = 3/group), and then the animals were returned to their home cages. The rats were decapitated 2 h after injection of the test drug or corresponding vehicle.

The brains were rapidly removed from the skull and were immediately frozen in dry ice-cooled 2-methylbutane (−40°C) and then stored at −80°C until sectioning. Twenty-micrometer-thick coronal frozen sections were cut using a cryostat microtome (Microm HM 500 O; Microm, Walldorf, Germany) and thaw-mounted on SuperFrost Plus microscope slides (Thermo Fisher Scientific). The sections were air-dried at room temperature and stored at −80°C until use.

Slides were brought to room temperature and immersed in 4% formaldehyde in phosphate-buffered saline (PBS, pH 7.4) for 30 min and then washed three times in PBS. The slides were placed in PBS containing 0.3% hydrogen peroxide for 30 min to quench endogenous peroxidases and washed three times with in TPBS (0.3% Triton X-100).
X-100 in PBS) with 1% (w/v) bovine serum albumin (BSA) fraction V (Sigma-Aldrich). Sections were then incubated in rabbit anti-c-fos serum (1:20,000, Ab-5; Calbiochem, San Diego, CA) in TPBS with 1% BSA overnight at 4°C, followed by three washes in TPBS with 1% BSA. Antigenic sites were visualized using a Vectastain Elite ABC kit (rabbit IgG) and 3,3-diaminobenzidine substrate kit for peroxidase (Vector Laboratories, Burlingame, CA). In brief, sections were incubated in biotinylated goat anti-rabbit IgG (1:1000) in TPBS with 1% BSA for 1 h at room temperature, followed by three washes in TPBS with 1% BSA. Sections were consecutively reacted with avidin-biotin peroxidase complex for 1 h and then washed in TPBS. The c-fos-immunoreactive nuclei were visualized with nickel-intensified 3,3-diaminobenzidine followed by three washes in water. The slides were then dehydrated using a series of graded ethanol, cleared in xylene, and coverslipped with Entellan New (Merck, Darmstadt, Germany). We used automated computer software (Image-Pro Plus, Media Cybernetics, Inc., Bethesda, MD) to count fos-like immunoreactivities in the brain sections.

**Data Analyses and Statistics.** Data analyses were performed using Prism software, version 4.00 (GraphPad Software Inc., San Diego, CA) and SAS software (SAS Institute, Cary, NC). One-way analysis of variance, followed by Dunnett’s test for multiple comparisons or Williams’ test, was used when appropriate. Values of $p < 0.05$ were considered statistically significant.

**Results**

In Vitro Profiles of CFMTI. Results are summarized in Table 1. In CHO cells expressing human mGluR1α and rat mGluR1α, CFMTI inhibited L-glutamate-induced increases in intracellular Ca$^{2+}$ concentrations, with IC$_{50}$ values of 2.6 ± 0.4 and 2.3 ± 0.3 nM, respectively. Concentration-response curve of CFMTI against human mGluR1 was shown in Fig. 1B. The IC$_{50}$ values of CFMTI against human mGluR5 were 5400 ± 1200 nM, showing that the activity of CFMTI is more than 2000-fold weaker against human mGluR5 than against human mGluR1. CFMTI up to 10 μM exhibited no agonistic activity toward any group I mGluR subtypes (data not shown). The selectivity of CFMTI was tested against group II and group III mGluR subtypes using the $[^{35}S]$GTP$_{Y}$S binding assay. CFMTI at 10 μM did not exhibit any agonistic or antagonistic activity against group II and group III mGluR subtypes, and there was no positive allosteric modulator activity on agonist-induced responses in these receptor subtypes (Supplemental Fig. 1). The selectivity of 10 μM CFMTI was further tested against 166 target molecules, such as an enzyme, neurotransmitter receptors, transporters, and ion channels, including ionotropic glutamate receptors (MDS Pharma, Bothell, WA; see Supplemental Table). The IC$_{50}$ values of CFMTI were higher than 10 μM against all targets, such as NMDA receptors (data not shown). To analyze the mode of action of CFMTI, the effects of CFMTI were examined using agonist concentration-response curves of intracellular Ca$^{2+}$ mobilization in CHO cells expressing human mGluR1α. Agonist concentration-response curves for L-glutamate-induced increases in intracellular Ca$^{2+}$ concentrations were generated in the absence or presence of CFMTI. The maximal response of L-glutamate was reduced in the presence of higher concentrations of CFMTI, supporting a noncompetitive antagonism (Supplemental Fig. 2). Finally, up to 10 μM CFMTI did not displace $[^{3}H]$-quisqualate bound to membranes from CHO cells expressing human mGluR1.

**Pharmacokinetic Study in Rats.** The plasma concentration of CFMTI was determined 5, 15, and 30 min and 1, 2, 4, 6, 8, and 24 h after intravenous or oral administration. After oral administration of CFMTI at a dose of 3 mg/kg, the maximal plasma concentration of CFMTI reached 4.8 ± 0.6 μM at 1.2 ± 0.5 h after dosing ($T_{\text{max}}$). The AUC$_{0-24\text{h}}$ and the oral bioavailability were calculated to be 20.5 ± 2.9 μM·h and 108.5 ± 15.1%, respectively (Supplemental Fig. 3).

**DHPG-Induced Face-Washing Behavior in Mice.** Intracerebroventricular administration of DHPG at 10 nmol/mouse elicited face-washing behavior. Oral administration of CFMTI inhibited DHPG-induced face-washing behavior in a dose-dependent manner (Fig. 2).

**Ex Vivo Receptor Occupancy Test.** CFMTI produced dose-dependent inhibition of specific ex vivo binding of $[^{3}H]$FTIDC to striatal and cerebellar slices in mice. Results are shown in Table 2. Occupancy levels of mGluR1 in the striatum and cerebellum were almost the same. Nearly 90% occupancy of mGluR1 was achieved by CFMTI at exposure levels of 0.9 ± 0.1 nmol/g and 1.9 ± 0.4 μM in brain and plasma, respectively, 60 min after oral administration of CFMTI at a dose of 30 mg/kg.

**Spontaneous Locomotor Activity under Nonhabituated Condition and MAP-Induced Hyperlocomotion in Mice.** CFMTI significantly inhibited hyperlocomotion induced by MAP at a dose of 2 mg/kg, without affecting spontaneous locomotor activity at up to 30 mg/kg (Fig. 3, A and D). Haloperidol significantly decreased MAP-induced hyperloco-

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**Fig. 2.** Effects of CFMTI on face-washing behavior produced by DHPG in mice. Data are presented as mean ± S.E.M. veh, vehicle. The number of mice used in each group is indicated in parentheses. ***, p < 0.01 versus vehicle-treated group; #, p < 0.05 and ##, p < 0.01 versus DHPG-treated group (one-way analysis of variance followed by Williams’ test).

**TABLE 1**

In vitro antagonistic activities of CFMTI on mGluR1 and other mGluRs, and affinity for the orthosteric glutamate binding site in mGluR1 using radiolabeled quisqualic acid

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<tr>
<th>mGluR</th>
<th>IC$_{50}$ (nM)</th>
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<tr>
<td>hmGluR1</td>
<td>2.6 ± 0.4</td>
<td>5</td>
</tr>
<tr>
<td>rmGluR1</td>
<td>2.3 ± 0.3</td>
<td>3</td>
</tr>
<tr>
<td>hmGluR5</td>
<td>5400 ± 1200</td>
<td>7</td>
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<tr>
<td>hmGluR2</td>
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<td>3</td>
</tr>
<tr>
<td>hmGluR4</td>
<td>&gt;10,000</td>
<td>3</td>
</tr>
<tr>
<td>hmGluR6</td>
<td>&gt;10,000</td>
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</tr>
<tr>
<td>hmGluR7</td>
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<td>3</td>
</tr>
<tr>
<td>hmGluR8</td>
<td>&gt;10,000</td>
<td>3</td>
</tr>
<tr>
<td>Quisqualic acid binding site</td>
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<td>4</td>
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motion in a dose-dependent manner at 0.1 and 0.3 mg/kg (Fig. 3B), with significant reduction of spontaneous locomotor activity at 0.3 mg/kg (Fig. 3E). Clozapine significantly attenuated MAP-induced hyperlocomotion at a dose range of 3 to 30 mg/kg. Clozapine alone caused significant hypolocomotion at a dose of 3 mg/kg (Fig. 3, C and F).

**Ketamine-Induced Hyperlocomotion under Habituated Condition in Mice.** Oral administration of CFMTI inhibited ketamine-induced hyperlocomotion at the dose range of 10 to 30 mg/kg (Fig. 4A). The effect of CFMTI at 30 mg/kg was statistically significant (p < 0.01). Subcutaneous administration of haloperidol significantly decreased ketamine-induced hyperlocomotion at 0.3 mg/kg (Fig. 4B). Subcutaneous administration of clozapine significantly attenuated ketamine-induced hyperlocomotion at 0.3 and 1 mg/kg (Fig. 4C).

**PPI in Rats.** CFMTI, haloperidol, and clozapine did not affect spontaneous PPI at all doses tested (Table 3). MAP (3 mg/kg s.c.) significantly disrupted PPI in rats at 6- and 12-dB prepulse intensity (Fig. 5, A–C). MAP-induced deficits in PPI were partially reversed by CFMTI at 10 mg/kg (Fig. 5A). Haloperidol reversed MAP-induced deficits in PPI at doses of 0.03 and 0.3 mg/kg (Fig. 5B). Likewise, clozapine improved PPI disruption induced by MAP at 3 to 30 mg/kg in a dose-dependent manner (Fig. 5C). In ketamine-induced PPI disruption, ketamine (5 mg/kg s.c.) significantly disrupted PPI in rats at 6- and 12-dB prepulse intensity (Fig. 6, A–C). CFMTI reversed ketamine-induced deficits in PPI only at a dose of 3 mg/kg (Fig. 6A). Ketamine-induced deficits in PPI seemed to be reversed by haloperidol; however, these differences did not reach statistical significance (Fig. 6B). Clozapine antagonized against PPI disruption induced by ketamine at 10 and 30 mg/kg (Fig. 6C).

**MK-801-Induced Deficits in Social Interaction in Rats.** Subcutaneous administration of MK-801 at 0.1 mg/kg significantly decreased total duration of social interaction between two naive rats compared with the vehicle-treated group. CFMTI significantly improved social withdrawal induced by MK-801 at 10 mg/kg, without affecting the number of line crossings during the observation period (Fig. 7, A and D). Haloperidol did not alter MK-801-induced social withdrawal at up to 1 mg/kg (Fig. 7B); however, the number of line crossings decreased significantly at 0.1 mg/kg (Fig. 7E). Clozapine attenuated MK-801-induced deficits in social in-

### Table 2

<table>
<thead>
<tr>
<th>Dose</th>
<th>mGluR1 Occupancy</th>
<th>Brain</th>
<th>Plasma</th>
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<tr>
<td></td>
<td>Striatum</td>
<td>Cerebellum</td>
<td>nmol/g</td>
<td>%</td>
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<tr>
<td>3 mg/kg p.o.</td>
<td>73 ± 7</td>
<td>70 ± 8</td>
<td>0.2 ± 0.07</td>
<td>0.2 ± 0.1</td>
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<tr>
<td>10 mg/kg p.o.</td>
<td>87 ± 1</td>
<td>82 ± 2</td>
<td>0.4 ± 0.04</td>
<td>0.6 ± 0.06</td>
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<tr>
<td>30 mg/kg p.o.</td>
<td>94 ± 1</td>
<td>89 ± 2</td>
<td>0.9 ± 0.1</td>
<td>1.9 ± 0.4</td>
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</tbody>
</table>

**Fig. 3.** Effects on methamphetamine-induced hyperlocomotion (A–C) and spontaneous locomotor activities (D–F). A and D, effects of CFMTI. B and E, effects of haloperidol. C and F, effects of clozapine. Data are presented as means ± S.E.M. veh, vehicle; HAL, haloperidol; and CLZ, clozapine. The number of mice used in each group is indicated in parentheses. *p < 0.05 and **p < 0.01 versus vehicle-treated group; #, p < 0.05 and ##, p < 0.01 versus methamphetamine-treated group (one-way analysis of variance followed by Dunnett’s test).
teraction at a dose of 3 mg/kg but did not change the number of line crossings (Fig. 7, C and F).

Catalepsy Assay in Rats. Administration of CFMTI or clozapine at up to 100 mg/kg did not cause any catalepsy, whereas haloperidol elicited catalepsy in a dose-dependent manner (Table 4).

Rotarod Test in Mice. CFMTI at doses of 3 to 100 mg/kg had negligible effects on motor coordination in the Rotarod test, whereas clozapine significantly impaired motor coordination at doses of 3 and 10 mg/kg (Fig. 8, A and B).

Effects of CFMTI on Object Location Memory in Mice. Oral administration of CFMTI at 10 mg/kg did not change location index in mice (Fig. 9). The approach time in each treated group was not different among groups (6.6 ± 1.7, 6.3 ± 1.1, and 7.4 ± 1.1 s for vehicle, 3 mg/kg CFMTI, and 10 mg/kg CFMTI, respectively).

Immunohistochemical Analysis of c-fos Expression in Rats. CFMTI, clozapine, and haloperidol significantly increased the number of fos-positive neurons in the nucleus accumbens (NAC, Figs. 10A and 11, B–E). In the dorsolateral
stratum (dlSTR), haloperidol significantly increased the number of fos-positive neurons, whereas CFMTI caused no increase (Figs. 10B and 11B and F–H). Although clozapine resulted in increased numbers of neurons expressing c-fos, the increase was not statistically significant (Fig. 10B). CFMTI and clozapine significantly increased the number of fos-positive neurons in the medial prefrontal cortex (mPFC), whereas haloperidol did not (Figs. 10C and 11A and I–K). Relatively high levels of fos-positive cell numbers in the control group for haloperidol treatment may be due to the different vehicle used in the haloperidol group.

Discussion

In this study, we demonstrated that a potent, selective, and orally active mGluR1 allosteric antagonist, CFMTI, improved not only MAP-induced behavioral alterations but also ketamine-induced hyperlocomotion and PPI disruption and MK-801-induced deficits in social interaction in rats. The effects of CFMTI were comparable with those of clozapine. The atypical antipsychotic-like effects of CFMTI were further demonstrated by that blockade of mGluR1 increased numbers of c-fos-expressing cells in NAC and mPFC but not in dlSTR.
In L-glutamate-induced intracellular Ca\(^{2+}\) mobilization assays, CFMTI showed similar antagonist activities in hmgluR1 and rmgluR1, with IC\(_{50}\) values of 2.6 ± 0.4 and 2.3 ± 0.3 nM, respectively. CFMTI noncompetitively decreased the maximal 1-glutamate-induced Ca\(^{2+}\) mobilization and did not displace \(^{3}H\)quisqualate binding in CHO-hmgluR1a, indicating that CFMTI was a negative allosteric modulator. CFMTI was more than 2000-fold more selective toward mGluR1 than mGluR5, and CFMTI showed no antagonistic, agonistic, or positive allosteric modulator activities toward other mGluR subtypes at concentrations up to 10 μM. In addition, CFMTI had no activities on iGluRs, other neurotransmitter receptors, ion channels, or transporters tested using binding or functional studies. These data indicate that CFMTI is a highly potent and selective allosteric mgluR1 antagonist with good oral bioavailability and is one of the best-in-class among existing mgluR1 antagonists, such as R214127, JNJ16259685, EMQMCM, and FTIDC in vitro (Mabire et al., 2005; Suzuki et al., 2007).

The antagonistic effect of CFMTI was tested against DHPG-induced face-washing behavior in mice, in which in vivo potencies of mgluR1 antagonists could be selectively evaluated (Hikichi et al., 2008). Oral administration of CFMTI at 30 mg/kg significantly suppressed face-washing behavior to 20% of that elicited by DHPG. At this dose, CFMTI occupied 94% of striatal mGluR1. Based on these results, 30 mg/kg CFMTI should be sufficient to obtain the maximum pharmacological effects by blocking mGluR1. CFMTI blocked MAP-induced hyperlocomotion and PPI disruption in the same manner as haloperidol and clozapine. It has been shown previously that amphetamines increase glutamate release in the ventral tegmental area and NAC (Xue et al., 1996; Wolf and Xue, 1999) and that administration of DHPG in the NAC disrupts PPI in rats (Grauer and Marquis, 1999). Therefore, the psychostimulant activity of amphetamines may, at least in part, be mediated by activation of mGluR1 by released glutamate in the basal forebrain and/or midbrain.

In addition to antagonism against MAP-induced abnormal behaviors, CFMTI significantly improved ketamine-induced hyperlocomotion and PPI disruption, which was also reversed by the atypical antipsychotic, clozapine, whereas a typical antipsychotic, haloperidol, only blocked ketamine-induced hyperlocomotion at 0.3 mg/kg, which reduced spontaneous locomotion. CFMTI did not cause catalepsy and motor incoordination at doses to demonstrate antipsychotic effects in various models. For haloperidol, a dose to antagonize MAP- and ketamine-induced hyperlocomotion caused catalepsy as well as motor incoordination (Satow et al., 2008). Clozapine caused motor incoordination without catalepsy at doses where antipsychotic effects were demonstrated. The effective doses of CFMTI in ketamine-induced PPI disruption were 3 and 10 mg/kg, at which striatal mgluR1 receptor occupancies were 73 and 87%, respectively. The same dose range of CFMTI was demonstrated to be effective in both DHPG-induced face-washing behavior and the MAP-induced PPI disruption model. The effects of mgluR1 antagonists on PPI disrupted by NMDA antagonists have not been fully elucidated. Improved effects of EMQMCM derivatives on ketamine-induced PPI disruption were demonstrated in an abstract form (Lesage et al., 2002). However, Spooren et al. (2003) introduced no effects of BAY 36-7620 on MK-801-induced PPI disruption as unpublished data. EMQMCM, studied only at 4 mg/kg, was shown to be ineffective in MK-801-induced PPI disruption (Pietraszek et al., 2005). Because the receptor occupancy profile of EMQMCM remains unclear, higher doses of EMQMCM should be investigated to explain these conflicting results.

MK-801-induced deficits in social interaction may be an animal model for negative symptoms of schizophrenia, particularly social withdrawal. In this test, CFMTI and clozapine improved MK-801-induced social withdrawal without affecting locomotor activities, indicating that positive effects of these drugs on social withdrawal are not due to apparent behavioral activation. Haloperidol did not improve MK-801-induced social withdrawal, even at doses causing significant hypolocomotion. The present finding with CFMTI is the first to demonstrate favorable effects of this mgluR1 antagonist in social deficits induced by MK-801. As described above, it is unlikely that the inhibitory effects of CFMTI on behavioral alterations elicited by NMDA antagonists are due to direct modulation of the NMDA receptor, because no affinity of CFMTI for these CNS targets was detected. The dosage of CFMTI used in the MK-801-induced social withdrawal test was identical to that used in the DHPG-induced face-washing test and in the ex vivo mgluR1 occupancy study, raising possibility that improving effects of CFMTI on MK-801-induced social withdrawal may be due to blockade of mgluR1 by CFMTI.

Because it has been demonstrated that acute administration of NMDA antagonists, such as phencyclidine (PCP), ketamine, and MK-801, facilitate release of glutamate in the prefrontal cortex in rodents (Moghaddam et al., 1997), increased extracellular levels of glutamate would be involved in NMDA antagonist-induced behavioral alteration such as PPI disruption and/or deficits in social interaction. Although enhanced glutamate release, hyperlocomotion, and stereotypy elicited by PCP were blocked by LY354740 (Moghaddam and Adams, 1998), improving effects of mgluR2/3 agonists on PCP-induced PPI disruption were limited (Schreiber et al., 2000; Henry et al., 2002; Galici et al., 2005, 2006). At present, we could not explain why mgluR2/3 agonists only reversed hyperlocomotion and stereotypy elicited by PCP but not PPI disruption. These results suggest that presynaptic control of the glutamatergic nervous system is apparently less signifi-

### Table 4

<table>
<thead>
<tr>
<th>Dose</th>
<th>Duration of Catalepsy</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>0.6 ± 0.1</td>
<td>11</td>
</tr>
<tr>
<td>CFMTI 30 mg/kg p.o.</td>
<td>2.0 ± 1.1</td>
<td>5</td>
</tr>
<tr>
<td>CFMTI 100 mg/kg p.o.</td>
<td>0.4 ± 0.1</td>
<td>5</td>
</tr>
<tr>
<td>Vehicle</td>
<td>2.9 ± 0.9</td>
<td>15</td>
</tr>
<tr>
<td>Haloperidol 0.03 mg/kg s.c.</td>
<td>1.4 ± 0.3</td>
<td>5</td>
</tr>
<tr>
<td>Haloperidol 0.1 mg/kg s.c.</td>
<td>7.0 ± 1.5</td>
<td>5</td>
</tr>
<tr>
<td>Haloperidol 0.3 mg/kg s.c.</td>
<td>45.0 ± 4.5**</td>
<td>10</td>
</tr>
<tr>
<td>Vehicle</td>
<td>1.6 ± 0.6</td>
<td>5</td>
</tr>
<tr>
<td>Clozapine 3 mg/kg s.c.</td>
<td>3.3 ± 2.2</td>
<td>5</td>
</tr>
<tr>
<td>Clozapine 10 mg/kg s.c.</td>
<td>7.5 ± 4.4</td>
<td>5</td>
</tr>
<tr>
<td>Clozapine 30 mg/kg s.c.</td>
<td>5.7 ± 3.5</td>
<td>5</td>
</tr>
<tr>
<td>Clozapine 100 mg/kg s.c.</td>
<td>5.1 ± 1.3</td>
<td>5</td>
</tr>
</tbody>
</table>

* *p < 0.01 versus corresponding vehicle control group.*
c-tant in PCP-induced PPI disruption. Blockade of postsynaptic mGluR5 by 2-methyl-6-(phenylethynyl)-pyridine or (2- methyl-1,3-thiazol-4-yl) ethynlpyridine was demonstrated to exacerbate NMDA antagonist-induced PPI disruption (Henry et al., 2002; Kinney et al., 2003; Pietraszek et al., 2005). Presynaptic mGluR5 seemed to release glutamate (Musante et al., 2008), which may further complicate the behavioral outcomes. Our previous work has suggested that postsynaptic blockade of mGluR1 and mGluR5 would produce opposite pharmacological effects and that presynaptic inhibition of glutamatergic neuronal activities by mGluR2/3 activation could result in sum of postsynaptic blockade of both mGluR1 and mGluR5, at least in the dopamine-dependent neuronal system (Satow et al., 2008). Thus, improving effects of CFMTI on NMDA antagonist-induced behavioral alterations are compatible with our working hypothesis, expanding the use of CFMTI to dopaminergic-independent neuronal control of psychiatric behavior. In support of this idea, an mGluR5 antagonist was reported to impair social interaction in rats (Koros et al., 2007). Melendez et al. (2005) reported that DHPG administered by reverse microdialysis into mPFC elicited glutamate release that was blocked by coapplication of (R,S)-1-aminoindan-1,5-dicarboxylic acid and LY367385 with DHPG, suggesting that mGluR1 in mPFC may play a role in regulating glutamate release. Because mGluR1 was localized on dendrites of pyramidal cells in mPFC (Muly et al., 2003), blockade of postsynaptic mGluR1 by CFMTI might suppress excess glutamate neurotransmission elicited by NMDA antagonists in the mPFC.

Because expression of the immediate early gene c-fos is increased by neuronal activation, induction of c-fos in neurons is a useful marker to map changes in neuronal activity (Ananth et al., 2001). Antipsychotics are known to induce c-fos in various brain regions. Among them, expression of c-fos in NAC might be related to some extent to the antipsychotic action of typical and atypical antipsychotics. In addition to c-fos induction in NAC, the atypical antipsychotic, clozapine, is known to induce c-fos in mPFC, whereas typical antipsychotics do not induce c-fos. In contrast, induction of c-fos in dSTR might be a response to extrapyramidal side effects of neuroleptics (Fink-Jensen and Kristensen, 1994; Wan et al., 1995). CFMTI significantly increased numbers of c-fos-positive cells in NAC as well as in mPFC, but not in dSTR, indicating that the antipsychotic-like effects of CFMTI are more similar to atypical one such as clozapine but not of haloperidol by some extent.

A hallmark of schizophrenia is cognitive impairment in addition to positive and negative symptoms, and treatments with antipsychotics improve cognitive dysfunction (Meltzer and McGurk, 1999). However, there are not many studies

\[ \text{Fig. 8.} \text{ Effects on rota-rod performance in mice.} \]
\[ \text{A, effects of CFMTI. B, effects of clozapine.} \]
\[ \text{Data are presented as means } \pm \text{ S.E.M. The number of mice used in each group is indicated in parentheses. Doses are in milligrams per kilogram. Some upper and lower error bars are omitted for clarity. } ** , p < 0.01 \text{ versus vehicle-treated group at each time point (one-way repeated measures analysis of variance followed by Dunnett’s test).} \]

\[ \text{Fig. 9.} \text{ Effects of CFMTI on object location memory in mice.} \]
\[ \text{Data are presented as means } \pm \text{ S.E.M. The number of mice used in each group is indicated in parentheses.} \]

\[ \text{Fig. 10.} \text{ Effects of CFMTI and antipsychotics on c-fos expression in neuronal cells.} \]
\[ \text{A, effects in the nucleus accumbens. B, effects in the dorsolateral striatum. C, effects in the medial prefrontal cortex.} \]
\[ \text{Data are presented as means } \pm \text{ S.E.M. The number of rats used in each group is indicated in parentheses. CFMTI was orally administered, clozapine was given by intraperitoneal administration, and haloperidol was subcutaneously administered. Doses are in milligrams per kilogram. veh, vehicle; HAL, haloperidol; and CLZ, clozapine. } * , p < 0.05 \text{ and } ** , p < 0.01 \text{ versus vehicle-treated group (one-way analysis of variance followed by Dunnett’s test).} \]
available to evaluate cognitive performance after treatments with antipsychotics in animal models. Some literature indicate that blockade of mGluR1 causes cognitive impairment; however, CFMTI did not impair spatial memory task at a dose that demonstrated antipsychotic activities in animal models (10 mg/kg). The present study did not address whether mGluR1 antagonists improved cognitive deficits caused by NMDA antagonists. Further study will be required if blockade of mGluR1 improve all aspects of schizophrenia, including cognitive deficits.

In summary, CFMTI has atypical antipsychotic-like activities in various animal models. CFMTI, similar to clozapine, blocked not only dopamine-related but also NMDA-related abnormal behaviors, which were considered to be relevant animal models for antipsychotics screening. CFMTI did not produce catalepsy, hypolocomotion, or motor incoordination. Increased c-fos expressions by CFMTI in NAC and mPFC, but not in dlSTR, were similar to those by clozapine. These results suggest that antipsychotic activities produced by blockade of mGluR1 may be similar to those of atypical antipsychotic drugs and that blocking mGluR1 could be a novel treatment for schizophrenia.

Acknowledgments
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