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Antipsychotic-like effects of a novel phosphodiesterase 10A inhibitor T-251 in rodents

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ABSTRACT

Phosphodiesterase 10A (PDE10A) is a dual-substrate PDE that hydrolyzes both cAMP and cGMP. PDE10A is selectively expressed in medium spiny neurons in the striatum, suggesting the potential of PDE10A inhibitors in the treatment of schizophrenia. This study presents the pharmacological profile of a novel PDE10A inhibitor, 2-[(E)-2-(7-fluoro-3-methylquinoxalin-2-yl)vinyl]-6-pyrrolidin-1-yl-N-(tetrahydro-2H-pyran-4-yl)pyrimidin-4-amine hydrochloride (T-251) in rodent models of schizophrenia. T-251 showed a potent inhibitory activity against human PDE10A (IC_{50} = 0.050 nmol/L) and showed high selectivity over other PDE families which have over 10,000-fold IC_{50} values. Oral administration of T-251 (0.1–1.0 mg/kg) increased cAMP and cGMP in the striatum in a dose-dependent manner. Oral administration of T-251 attenuated MK-801 induced hyperactivity (ED_{50} = 0.68 mg/kg) and suppressed conditioned avoidance response (ID_{50} = 0.87 mg/kg) in rats in a dose dependent manner. Furthermore, T-251 significantly attenuated MK-801 induced prepulse inhibition deficits and cognitive deficits in rats. Unlike haloperidol and olanzapine, T-251 (1.0–30 mg/kg) did not cause catalepsy in rats. Moreover, T-251 (0.6 and 6.0 mg/kg) did not increase plasma levels of prolactin at 1 h after administration, whereas haloperidol and olanzapine significantly increased them. The antipsychotic-like effects and cognitive enhancement of T-251 without catalepsy or plasma prolactin elevation observed in rats suggests that T-251 would be a novel antipsychotic with an improved side-effect profile.

1. Introduction

Schizophrenia is a major psychiatric disorder characterized primarily by positive symptoms, negative symptoms and cognitive impairment. The current antipsychotics are full antagonists and partial agonists of dopamine D_2 receptors; however, the efficacy of current antipsychotics are still not satisfactory (Citrome, 2014; Corponi et al., 2019; Forray and Buller, 2017). Although atypical antipsychotics could reduce the risk of extrapyramidal symptoms (EPS) of typical antipsychotics, efficacy is considerably limited for negative symptoms and cognitive impairment (Forray and Buller, 2017; Serretti et al., 2004). In addition, atypical antipsychotics are also associated with hyperprolactinemia, osteoporosis possibly induced by hyperprolactinemia, and serious metabolic side effects, including hyperglycemia, weight gain, and diabetes (De Hert et al., 2016; Krebs et al., 2006; Serretti et al., 2004; Tschoner et al., 2007). Recently, long-acting injectable antipsychotics are reported to be more effective for cognitive impairment (Correll et al., 2016; Guillena and Garcia de Diego, 2019), but it is still necessary to develop new drugs that are more convenient and effective in the treatment of negative symptoms and cognitive impairment in schizophrenia.

Phosphodiesterase 10A (PDE10A), a dual hydrolase of cAMP and cGMP, is highly expressed in medium spiny neurons (MSNs) in the striatum (Fujishige et al., 1999a,b; Seeger et al., 2003; Soderling et al., 1999; Xie et al., 2006). One of the substrates of PDE10A, cAMP, is the major second messenger downstream of G protein-coupled receptors, including Gs-coupled dopamine D_1 receptor and Gi-coupled D_2 receptor (Beaulieu et al., 2015). Inhibition of PDE10A induces elevation of cAMP, and subsequently activates cAMP/protein kinase A (PKA) signaling in both D_1- and D_2-type neurons with an agonist-like effect against D_1 receptor and an antagonist-like effect against D_2 receptor. Activation of both D_1-type neurons and D_2-type neurons are expected to reduce the risk of EPS retaining the antipsychotic effects by balancing the striatal total output. Furthermore, activation of MSNs in the

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striatum, where cortical glutamatergic and midbrain dopaminergic inputs are integrated, could recover a hypoglutamatergic state-induced weakened striatal output which seems to be related with cognitive impairment and negative symptoms (Kehler and Nielsen, 2011; Snyder and Vanover, 2014). Thus, PDE10A inhibition has attracted attention as a new therapeutic approach which satisfies present unmet needs of schizophrenia.

In the last decade, several PDE10A inhibitors [*i.e.*, MP-10 (PF-02545920), TAK-063 (Balipodect), PDM-042] have been discovered as candidates of antipsychotics (Arakawa et al., 2016; Geerts et al., 2017; Grauer et al., 2009; Kehler, 2013; Kehler and Nielsen, 2011; Snyder and Vanover, 2017; Suzuki et al., 2015; Zagorska et al., 2018). A number of preclinical studies have demonstrated that the inhibition of PDE10A is effective not only for positive symptoms but also for negative symptoms and cognitive deficits in rodents (Arakawa et al., 2017; Grauer et al., 2009; Langen et al., 2012; Shiraishi et al., 2016). Importantly, PDE10A inhibitors have much less side effects than the current antipsychotics in rodents (Arakawa et al., 2016; Grauer et al., 2009; Suzuki et al., 2015).

Recently, we reported the discovery of a novel PDE10A inhibitor, 2-[(E)-2-(7-fluoro-3-methylquinoxalin-2-yl)vinyl]-6-pyrrolidin-1-yl-N-(tetrahydro-2H-pyran-4-yl)pyrimidin-4-amine hydrochloride (compound 32, T-251) (Kadoh et al., 2018). In the present study, we report the antipsychotic-like effects and side effect profiles of T-251 in rodents.

2. Materials and methods

2.1. Animals

Male C57/BL 6Cr mice (aged 7 weeks, body weight 20.3–25.8 g, Japan SLC, Inc., Hamamatsu, Japan) were used for the brain cAMP/cGMP study. Male Wistar rats (Japan SLC, Inc., Hamamatsu, Japan) were used for the locomotor activity study (aged 7–8 weeks, body weight 144–249 g), prepulse inhibition (PPI) test (aged 8 weeks, body weight 177–219 g) and prolactin measurement (aged 6 weeks, body weight 120–140 g). Male Wistar rats (Japan Charles River Inc., Yokohama, Japan) were used for the conditioned avoidance response (CAR) test (aged 8–12 weeks, body weight 260–437 g) and the catalepsy test (aged 7–8 weeks, body weight 147–195 g). Male Listar hooded rats (aged 7–8 weeks, body weight 208–286 g, Kyudo Co., Ltd., Saga, Japan) were used for the novel object recognition test (NORT).

All animals were housed under environmentally controlled conditions; 12-h light/dark cycle (lights on at 7:00 AM), setting temperature (permissive range): 23 (20–26)°C, setting humidity (permissive range): 55 (30–70)% with access to food and water *ad libitum*. All *in vivo* experimental procedures were approved by the Institutional Animal Care and Use Committee of Research Laboratories, Mitsubishi Tanabe Pharma Corporation.

2.2. Test compounds

T-251 and its 2 HCl salt (used in the catalepsy test) were synthesized at Mitsubishi Tanabe Pharma Corporation (Yokohama, Japan). T-251 was dissolved in dimethyl sulfoxide (DMSO) for the *in vitro* PDE assay or suspended in 0.5% (hydroxypropyl)methyl cellulose (HPMC)–0.1% Tween 80 (vehicle) for *in vivo* studies. As reference drugs, haloperidol was purchased from Sigma-Aldrich, Inc. (St. Louise, MO, USA), olanzapine and clozapine were synthesized at Mitsubishi Tanabe Pharma Corporation (Yokohama, Japan). All reference drugs were suspended in the same vehicle. Briefly, effects of T-251 were evaluated at 1 h after oral administration in *in vivo* studies in consideration of convenience for clinical use. All reference drugs were evaluated at the same time point of T-251. (+)-MK-801 hydrogen maleate (MK-801: Sigma-Aldrich, Inc., St. Louise, MO, USA) was dissolved in saline.

2.3. In vitro PDE assay

PDE1, PDE3, PDE4, PDE5 and PDE6 were isolated by chromatography of rat ventricle, dog heart, dog lung, dog lung and bovine retina, respectively, according to the method modified from a previous report (Kotera et al., 2000). Human recombinant PDE2A, PDE7B, PDE8A, PDE9A, PDE10A and PDE11A were obtained from COS-7 cells transfected with expression plasmids coded for each PDE subtype according to a method modified from previous reports (Gamanuma et al., 2003; Kotera et al., 1999; Sasaki et al., 2000; Yuasa et al., 2000).

The PDE assay was performed by the radio-labeled nucleotide method (Thompson et al., 1979). Briefly, T-251, enzymes, substrates (approximate 40 nmol/L [5′,8-^{3}H]cAMP or 163 nmol/L [8-^{3}H]cGMP: GE Healthcare Bio-Sciences, Pittsburgh, PA, USA) and 0.2 mg/mL snake venom were mixed in assay buffer (50 mmol/L Tris-HCl, pH 8.0, 12.5 mmol/L $MgCl_2$, 10 mmol/L 2-mercaptoethanol and 0.825 mg/mL bovine serum albumin) and incubated at room temperature for 90 min. The reaction was stopped by adding an equivalent volume of methanol. Resultant solutions were applied to a Dowex (1 × 8 200–400) resin filter and then washed with methanol. The radioactivity of eluates was measured with a scintillation counter (TopCount NXT™: Packard Instrument Company, Inc., Meriden, CT, USA). The IC_{50} values were calculated by non-linear regression using logarithmic concentrations and % inhibition.

2.4. Measurement of striatal cAMP and cGMP level

One hour after oral administration of T-251 (0.1, 0.3 or 1.0 mg/kg), mice were sacrificed by focused microwave irradiation to the brain. The striatum was isolated and homogenized in 0.5% Triton-X-100/phosphate buffered salts (PBS) and centrifuged at 21,880*g* at 4 °C for 5 min. The concentrations of cAMP and cGMP in supernatants were measured using enzyme immunoassay (EIA) kits (Cat. No. RPN225 and RPN 226, GE Healthcare UK Limited, Buckinghamshire, UK).

2.5. Locomotor activity

Locomotor activity of rats were measured in Plexiglas boxes (45 × 45 × 25 cm) set in the SCANET system (MV-20plus®, MELQUEST Ltd., Toyama, Japan), equipped with infrared photocells on the X and Y-axes. T-251 (0.09, 0.3, 1.0 or 3.0 mg/kg) or vehicle was orally administered to rats 1 h prior to measurements. For measurement of MK-801 induced hyperactivity, MK-801 (0.075 mg/kg) was subcutaneously injected just before the measurement. Rats were placed individually in the apparatus for 20 min for spontaneous activity tests and for 40 min for MK-801 induced hyperactivity. Total counts were measured, and ED_{50} values and their 95% confidence intervals (CI) were estimated by non-liner regression analysis.

2.6. CAR test

Conditioned avoidance behavior was assessed using automated shuttle-boxes (56 × 21 × 25 cm, MSB-001, MELQUEST Ltd., Toyama, Japan) each placed in a sound-attenuated chamber. The boxes were subdivided into two compartments by a partition with one opening. The position of rats and their movement from one compartment to the other were detected by photocells sensitive to infrared light on each side of the dividing wall.

Rats were trained and tested in the automated shuttle-boxes. A session consisted of 20 trials with 10 s inter-trial intervals. Each trial consisted of a 5 s tone (conditioned stimulus, CS) followed by a 10 s tone plus mild electric stimulus (0.8 mA, unconditioned stimulus, UCS) being applied to the grid floor. If the rat moved from one compartment to the other, the stimulus was immediately terminated. The following behavioral variables were recorded: avoidance (response to CS within 5 s), escape failures (failure to respond to UCS). Only rats that showed a

high level of avoidance response (at least 80%) on two or more consecutive days were used for evaluation of T-251. On the test day, rats were orally administered with T-251 (0.3, 1.0 or 3.0 mg/kg) or vehicle and tested 1, 3, 5, 7 and 24 h later as described above. Avoidance response (%) and escape failure (%) at each time point was calculated. The ID_{50} values and its 95% CI for avoidance response at each time point were estimated by non-liner regression analysis.

2.7. PPI test

PPI tests were conducted using four SR-LAB® acoustic startle chambers (San Diego Instruments, San Diego, CA, USA), each consisting of a clear, nonrestrictive Plexiglas cylinder mounted on a platform and housed in ventilated, sound-attenuating external chambers. Rats placed inside the cylinders are stimulated with acoustic stimuli, triggering the whole-body startle response, which is detected by transducing movement into analog signals by a piezoelectric unit attached to the platform. A loudspeaker inside each chamber provided a continuous background noise of 70 dB. The session consists of five different trials; (1) PULSE ALONE trial (40 milliseconds, 120 dB), (2–4) PREPULSE trial (20 millisecond prepulses at 80, 85 or 90 dB and 40 milliseconds PULSE 100 milliseconds after prepulse) and (5) NOSTIM trial (only the background noise) conducted in a pseudorandom order with an interval of 12–18 s. Each trial was conducted 12 times. The startle responses were averaged excluding the first time of each trial. PPI was calculated using the following formula: PPI (%) = 100 − [{(PREPULSE-NOSTIM) / (PULSE ALONE-NOSTIM)} ∗ 100]. On the day before the test, all rats underwent the baseline session. Rats that showed 10–299 responses in the PULSE ALONE trial and > 40% PPI (the average of PPI at 80, 85 and 90 dB) were used for the test session. In the test session, T-251 (1.0, 3.0 or 10 mg/kg) or vehicle were orally administered 1 h before the start of the test. MK-801 (0.075 mg/kg) was subcutaneously injected 25 min before the start of the test.

2.8. NORT

The experiments were carried out in a test box (45.5 × 25 × 35 cm) set in the SCANET system (MV-10AQ®, MATYS Co., Tokyo, Japan), equipped with infrared photocells on the X and Y-axes. Two objects; a blue plastic cube (7 × 6 × 6 cm) and a white porcelain pyramid (6 × 6 × 6.5 cm) were positioned in two adjacent corners in-touch of the walls of the test box.

Rats were habituated to the test box for 5 min on the day before the test day. On the test day, T-251 (0.2 or 0.6 mg/kg), clozapine (3.0 mg/kg) or vehicle were orally administered 1 h prior to intraperitoneal injection of MK-801 (0.05 mg/kg). The training session was conducted 10 min after MK-801 injection where rats were exposed to two identical objects for 5 min. The test session was conducted 2 h after the training session where rats were exposed to two objects for 5 min with one object replaced by a novel one. Object exploration was defined as the rats sniffing, licking, touching or biting. Exploration preference was calculated using the following formula: Exploration preference (%) = (time spent exploring the novel object) / (the total exploration time of the two objects) × 100

2.9. Catalepsy

Rats were orally administered with T-251 or vehicle and tested 1, 3, 5 and 7 h later for catalepsy by the bar test. Forepaws of the rats were placed on a 7 cm-high bar and recorded for how long they retained the same position. Rats were removed from the bar if their latency on the bar exceeded 60 s (*i.e.* cut-off time = 60 s). The effective dose of 10 s (ED_{10s} value) and its 95% CI for each compound was determined from the sum of total catalepsy time from 4 trials, to assess potency of the test compounds to induce catalepsy. ED_{10s} were estimated by linear regression analysis.

2.10. Plasma prolactin level

Rats were sacrificed by decapitation under non-anesthesia at 1 h after oral administration of the test compounds. Trunk blood was collected and centrifuged at 1600 ×*g* at 4 °C for 20 min, and the supernatant was collected as plasma samples. The plasma prolactin concentration was determined using an EIA kit (RAT PROLACTIN ENZYME IMMUNOASSAY KIT A05101-96 Wells, SPI-bio Inc., France).

2.11. Statistical analysis

Data are expressed as mean ± standard error of the mean (S.E.M.). All statistical analyses were performed with SAS software (SAS Institute, Inc., Cary, NC, USA). For single measurement data, striatal cAMP and cGMP levels, total count of locomotor activity and plasma prolactin level, differences between the vehicle group and test compound-treated groups were compared by Dunnett's multiple comparison test. For the CAR test, differences between the vehicle group and T-251 treated groups were compared using repeated measures analysis of variance (ANOVA) followed by Dunnett's multiple comparison test at each time point. For the PPI test, comparisons between the normal control (vehicle + saline) group and the deficit control (vehicle + MK-801) group or between the deficit control group and T-251 treated groups were performed by two-way ANOVA followed by Student's *t*-test or Dunnett's multiple comparison test. For NORT, comparisons between the normal control group and the deficit control group or between the deficit control group and T-251 treated groups were performed by Student's *t*-test or Dunnett's multiple comparison test, respectively. The effect of clozapine, as a reference, was detected by Student's *t*-test with comparison to the deficit control group. Both the training session and the test session were subjected to statistical analyses. All statistical analyses were performed using a two-tailed test with a significance level of 0.05.

3. Results

3.1. PDE selectivity

T-251 showed a potent inhibitory effect against PDE10A with an IC_{50} value (95% CI) of 0.050 (0.038–0.067) nmol/L and showed high selectivity over other PDE families which had over 10,000-fold IC_{50} values (Table 1). These results indicate that T-251 is a potent and selective PDE10A inhibitor.

3.2. Effects of T-251 on striatal cAMP and cGMP levels

To investigate oral bioactivity of T-251 for clinical application, striatal cAMP and cGMP levels were measured in mice after oral administration of T-251. T-251 (0.1, 0.3 or 1.0 mg/kg) increased both cAMP and cGMP levels in a dose dependent manner (Fig. 1A and B). High dose (1.0 mg/kg) of T-251 significantly increased both cAMP

Table 1
PDE selectivity of T-251.

Subtypes	Source of enzymes	Substrates	IC_{50} (nmol/L)
PDE1	Rat ventricle	cGMP	> 10,000
PDE2A	Human recombinant	cGMP	> 10,000
PDE3	Canine heart	cAMP	> 10,000
PDE4	Canine lung	cAMP	1700
PDE5	Canine lung	cGMP	2100
PDE6	Bovine retina	cGMP	1900
PDE7B	Human recombinant	cAMP	> 10,000
PDE8A	Human recombinant	cAMP	> 10,000
PDE9A	Human recombinant	cGMP	> 10,000
PDE10A	Human recombinant	cAMP	0.050
PDE11A	Human recombinant	cGMP	4900

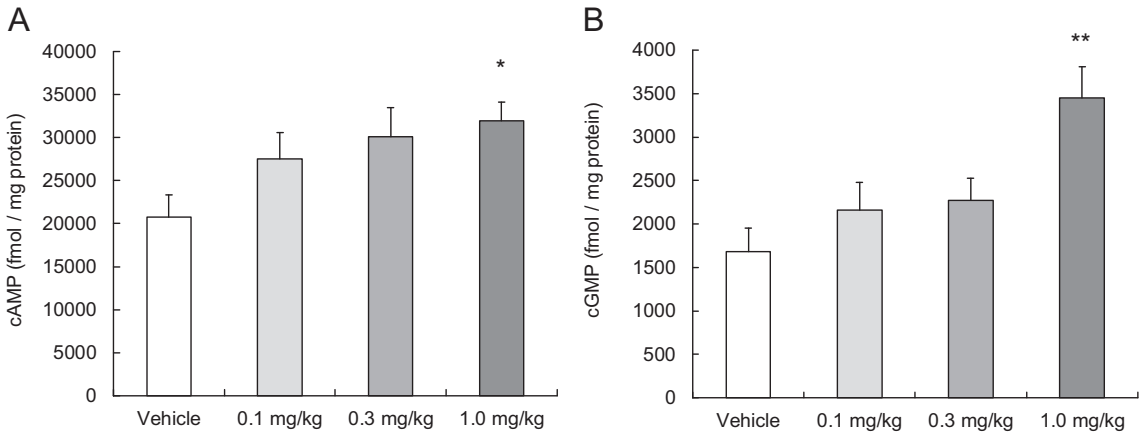

Fig. 1. Effects of T-251 on the cAMP and cGMP level in the mouse striatum.
Mice were sacrificed by focused microwave irradiation to the brain at 1 h after oral administration of T-251 (0.1, 0.3 or 1.0 mg/kg) or vehicle. (A) Striatal cAMP levels were determined using EIA kit. (B) Striatal cGMP levels were measured using EIA kit. Data are expressed as mean ± S.E.M. ($n = 7$ or 8). *$P < 0.05$, **$P < 0.01$ compared with the vehicle group (Dunnett's multiple comparison test).

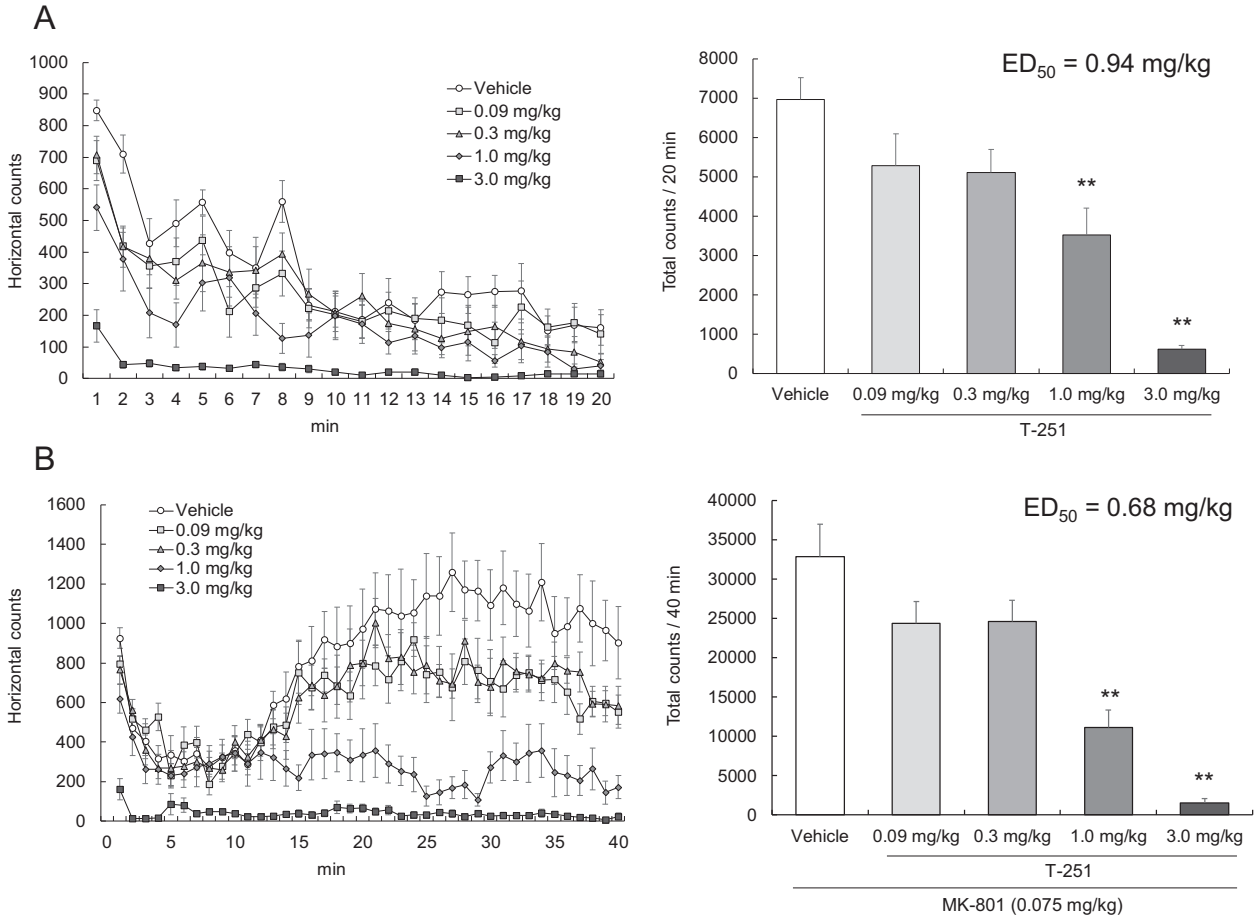

Fig. 2. Effects of T-251 on spontaneous locomotor activity and MK-801 induced hyperactivity in rats.
(A) Time-course and accumulative counts of spontaneous locomotor activity were measured for 20 min from 1 h after oral administration of T-251 (0.09, 0.3, 1.0 or 3.0 mg/kg) or vehicle. Locomotor activity was measured as described in the method. (B) Time-course and accumulative counts of locomotor activity for 40 min from just after injection of MK-801 (0.075 mg/kg, s.c.) 1 h after oral administration of T-251 (0.09, 0.3, 1.0, or 3.0 mg/kg) or vehicle. Data are expressed as mean ± S.E.M. ($n = 8$). **$P < 0.01$ compared with the vehicle group (Dunnett's multiple comparison test).

($P < 0.05$) and cGMP levels ($P < 0.01$) in the striatum (Fig. 1A and B).

3.3. Effects of T-251 on spontaneous locomotor activity and MK-801 induced hyperactivity

Oral administration of T-251 (0.09, 0.3, 1.0 or 3.0 mg/kg) decreased the spontaneous locomotor activity in rats in a dose dependent manner

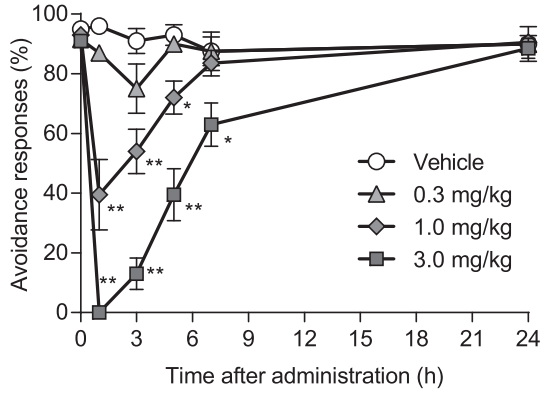

Fig. 3. Inhibitory effects of T-251 on CAR in rats.
The test sessions were conducted at 1, 3, 5, 7 and 24 h after the oral administration of T-251 (0.3, 1.0 or 3.0 mg/kg) or vehicle. Data are expressed as mean ± S.E.M. ($n = 10$). $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the vehicle group (Repeated measures ANOVA followed by Dunnett's multiple comparison test).

Table 2
Escape failure on each time point in CAR test for T-251.

	Escape failures (%)					
	Pre	1 h	3 h	5 h	7 h	24 h
Vehicle	0	0	0	0	0	0
0.3 mg/kg	0	0	0	0	0	0
1.0 mg/kg	0	10.5 ± 5.6	0	0	0	0
3.0 mg/kg	0	40.5 ± 9.9	14.0 ± 5.8	4.0 ± 3.1	0	0

Data are expressed as the mean ± S.E.M. ($n = 10$).

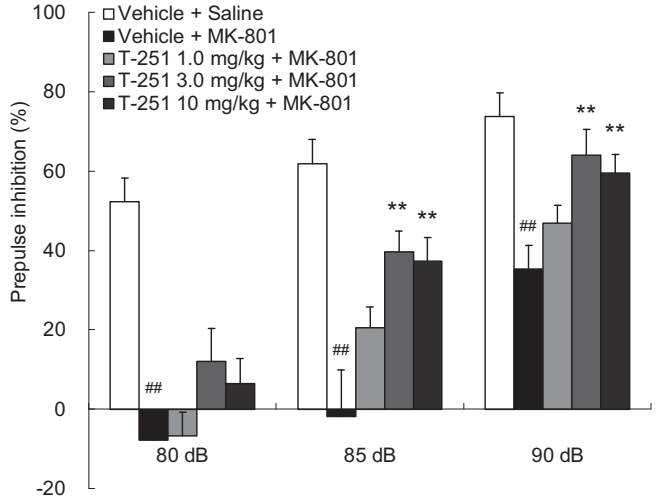

Fig. 4. Effect of T-251 on MK-801 induced PPI deficits in rats.
PPI at three different prepulse intensity levels (80, 85 and 90 dB). Rats were orally administrated T-251 and subcutaneously injected MK-801 (0.075 mg/kg), 1 h and 25 min before the test starts, respectively. Data are expressed as mean ± S.E.M. ($n = 10$ or 11). $^{\#\#}P < 0.01$ compared with the normal control (vehicle + saline) group (two-way ANOVA followed by Student's *t*-test), $^{**}P < 0.01$ compared with the deficit control (vehicle + MK-801) group (two-way ANOVA followed by Dunnett's multiple comparison test).

(Fig. 2A). The ED_{50} value (95% CI) of T-251 for spontaneous locomotor activity was 0.94 (0.52–1.7) mg/kg. T-251 (1.0 and 3.0 mg/kg) significantly ($P < 0.01$) decreased locomotor activity in rats (Fig. 2A). Furthermore, oral administration of T-251 (0.09, 0.3, 1.0 or 3.0 mg/kg) attenuated MK-801 induced hyperactivity in rats in a dose dependent

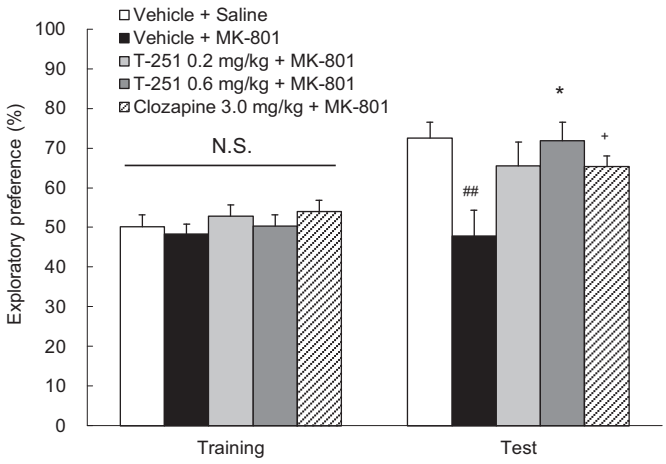

Fig. 5. Effect of T-251 on MK-801 induced cognitive deficits in NORT in rats.
Rats were orally administrated the test compounds (T-251 and clozapine, as the reference) 1 h prior to intraperitoneal injection of MK-801 (0.05 mg/kg). The training session was conducted 10 min after MK-801 injection and interval between the training session and the test session was 2 h. Data are expressed as mean ± S.E.M. ($n = 10$ or 11). $^{\#\#}P < 0.01$ compared with the normal control group (Student's *t*-test), $^{*}P < 0.05$ (Dunnett's multiple comparison test) and $^{+}P < 0.05$ (Student's *t*-test), as compared with the deficit control group. N.S.: No significant differences were detected in the training session.

manner (Fig. 2B). The ED_{50} value (95% CI) of T-251 for MK-801 induced hyperactivity was 0.68 (0.40–1.2) mg/kg. T-251 (1.0 and 3.0 mg/kg) significantly ($P < 0.01$) decreased MK-801 induced hyperactivity in rats (Fig. 2B).

3.4. Efficacy of T-251 in CAR test

The effect of T-251 (0.3, 1.0 or 3.0 mg/kg) in the CAR test was investigated. To assess the changes of efficacy over time, the test was conducted at multiple time points within 24 h. Oral administration of T-251 suppressed CAR in rats in a dose dependent manner (Fig. 3). Repeated measures ANOVA revealed the significant effect of T-251 (group: $F_{3,36} = 36.81$, $P < 0.01$; group × time: $F_{15,180} = 16.16$, $P < 0.01$). The inhibitory effect of T-251 on CAR peaked at 1 h after oral administration and decreased in a time-dependent manner. The ID_{50} values (95% CI) of T-251 were 0.87 (0.67–1.1), 1.2 (0.82–1.8) and 2.4 (1.7–3.4) mg/kg at 1, 3, 5 h after administration, respectively. At higher doses exceeding ID_{50} values for each time point, T-251 showed increase of escape failures (Table 2). The largest percentage of escape failures (40.5 ± 9.9%) was seen at 1 h after administration of T-251 (3.0 mg/kg).

3.5. Effect of T-251 on MK-801 induced PPI deficits in rats

The effects of T-251 (1.0, 3.0 and 10 mg/kg) on MK-801 induced PPI deficits in rats was investigated. Two-way ANOVA revealed the significant difference between the normal control group and the deficit control group (group: $F_{1,18} = 44.56$, $P < 0.01$, prepulse intensity: $F_{2,36} = 16.32$, $P < 0.01$, group × prepulse intensity: $F_{2,36} = 2.69$, $P = 0.08$) and significant effect of T-251 (group: $F_{3,38} = 6.73$, $P < 0.01$, prepulse intensity: $F_{2,76} = 97.18$, $P < 0.001$, group × prepulse intensity: $F_{6,76} = 1.21$, $P = 0.31$). By following *post-hoc* test at each prepulse intensity, T-251 (3.0 and 10 mg/kg) significantly attenuated MK-801 induced PPI deficits at 85 dB and 90 dB ($P < 0.01$), but not 80 dB (Fig. 4).

3.6. Effect of T-251 on MK-801 induced cognitive deficits in rats

In the training session, there were no statistical differences among

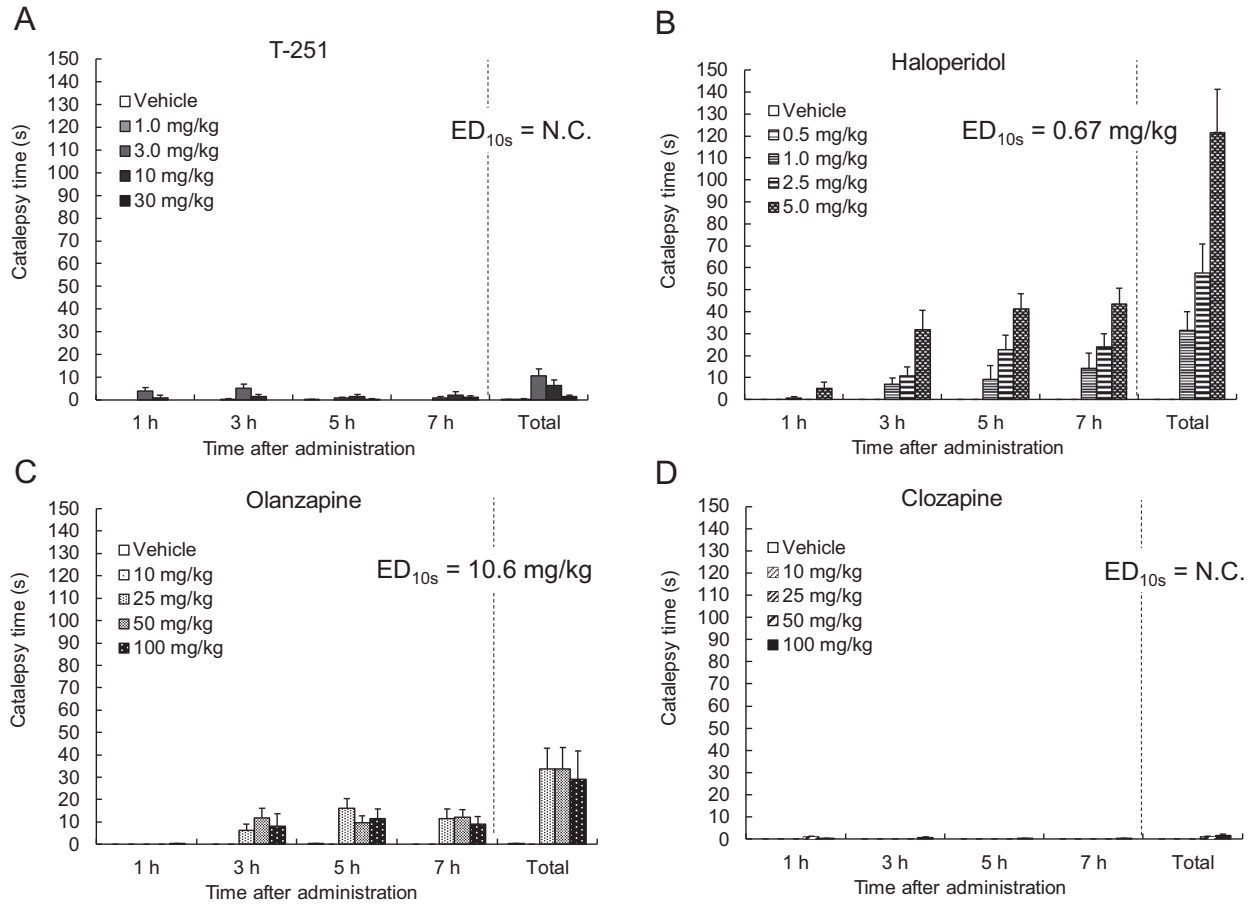

Fig. 6. Effects of T-251 (A), haloperidol (B), olanzapine (C) and clozapine (D) on cataleptogenic activity in rats.
Catalepsy time was measured by the bar test (7-cm height, cut-off time = 60 s). Measurement points were 1, 3, 5 and 7 h after the oral administration of the test compounds. Data are expressed as mean ± S.E.M. (n = 8). ED_{10s} were calculated from total catalepsy time using liner regression analysis. N.C.: not calculated.

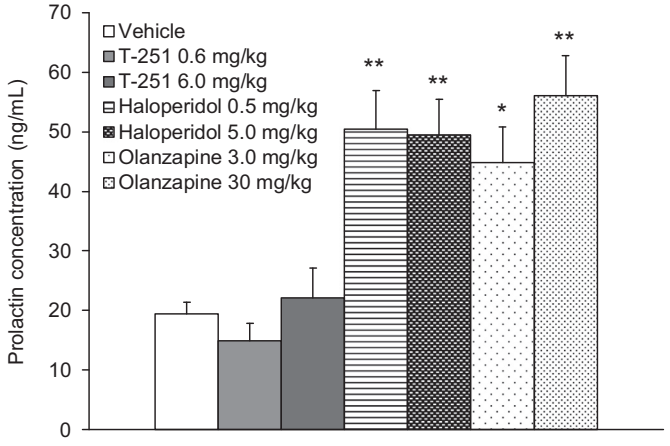

Fig. 7. Effect of T-251, haloperidol and olanzapine on plasma prolactin levels in rats.
Rats were sacrificed after 1 h after oral administration of the test compounds. The plasma prolactin concentrations were measured using EIA kit. Data are expressed as mean ± S.E.M. (n = 5). *P < 0.05, **P < 0.01 compared with the vehicle group (Dunnett's multiple comparison test).

all groups. Similar to clozapine (3.0 mg/kg), T-251 (0.6 mg/kg) significantly (P < 0.05) attenuated MK-801 induced cognitive deficits in rats (Fig. 5).

3.7. Effect of T-251 on catalepsy

To assess the potency of T-251 for extrapyramidal side effects, the catalepsy test was performed (Fig. 6). Haloperidol, olanzapine and clozapine were used as reference drugs. All drugs were evaluated at excess doses rather than their pharmacological effective doses; the highest dose was set at 5-fold or more than each ED_{50} value in the rat CAR test (data of reference drugs are not shown). Since typical antipsychotics were reported to elicit persistence catalepsy after acute administration in rats (Gyertyan et al., 2011; Morimoto et al., 2002), the test was conducted up to 7 h. T-251 (1.0–30 mg/kg) did not elicit catalepsy, whereas haloperidol and olanzapine elicited longer catalepsy, with ED_{10s} values (95% CI) of 0.67 (0.41–0.91) mg/kg and 10.6 (0.0000024–24.5) mg/kg, respectively. Contrastively, clozapine (10–100 mg/kg) did not induce catalepsy in rats. In this study, we did not calculate ED_{10s} values of T-251 and clozapine.

3.8. Effect of T-251 on plasma prolactin levels

Treatment with antipsychotics can be associated with hyperprolactinemia, which may be associated with a wide variety of side effects. To assess the potency of T-251 on induction of hyperprolactinemia, plasma levels of prolactin were measured (Fig. 7). Haloperidol and olanzapine were used as reference drugs. All drugs were evaluated at a pharmacological effective dose and a 10-fold higher dose. Haloperidol and olanzapine showed significant increase in plasma prolactin levels at 1 h after a single oral administration. In contrast, T-251 (0.6 and 6.0 mg/kg) did not increase plasma levels of prolactin in rats.

4. Discussion

In the last decade, clinical trials for several PDE10A inhibitors in patients with schizophrenia have been conducted. In a proof-of-concept (POC) study, MP-10 (PF-02545920) did not show beneficial effects compared to placebo (DeMartinis et al., 2019). Another PDE10A inhibitor TAK-063 (Balipodect) also did not achieve its primary endpoint (statistically significant difference in PANSS total score change compared with placebo at 6 weeks of treatment). However, some evaluation scales were ameliorated by TAK-063 (Macek et al., 2019), suggesting the efficacy of PDE10A inhibitors in schizophrenia could be demonstrated by stratifying patients. In both POC studies, although adverse events such as dystonia, akathisia and somnolence occurred, they were mild or moderate. Interestingly, TAK-063 showed fast off-rate from PDE10A compared to MP-10, and exerted different activation balances for D_1-type and D_2-type neurons (Suzuki et al., 2016; Suzuki and Kimura, 2018). These data suggest that PDE10A inhibitors with different off-rates may have different profiles for efficacy and adverse effects.

In this study, we demonstrated the pharmacological profile of T-251, a PDE10A inhibitor with a different chemical structure from MP-10 or TAK-063 (Kadoh et al., 2018) in rodents. T-251 showed significant antipsychotic-like effects with less side effects seen by dopamine D_2 receptor antagonists, similar to MP-10 and TAK-063.

T-251 showed potent inhibitory activity of PDE10A and high selectivity over other PDE families which have IC_{50} ratios of over 10,000 compared to PDE10A. An oral single administration of T-251 (1.0 mg/kg) significantly increased cAMP and cGMP levels in the striatum, indicating that T-251 causes PDE10A inhibition in the striatum after oral administration.

(+)-MK-801 has been used to cause schizophrenia-like behavioral abnormalities in rodents (Cadinu et al., 2018; Gobira et al., 2013). In this study, the ED_{50} value of T-251 for MK-801 induced hyperactivity was 0.68 mg/kg (p.o.). In contrast, T-251 significantly attenuated spontaneous activity in rats. Since the ED_{50} value of T-251 for spontaneous activity was 0.94 mg/kg, it seems that T-251 induced sedation may, in part, contribute to attenuation of MK-801 induced hyperlocomotion by T-251. The CAR test is widely used to predict antipsychotic-like effects in humans since the potency of dopamine D_2 blocking antipsychotics observed in the CAR test correlates well with antipsychotic effects in humans (Wadenberg, 2010). T-251 showed the highest efficacy at 1 h after oral administration with an ID_{50} value of 0.87 mg/kg. The effect of T-251 continued for up to 5 h. In addition, T-251 (3.0 and 10 mg/kg, p.o.) significantly attenuated MK-801 induced PPI deficits in rats although these doses of T-251 were higher than the ED_{50} (0.68 mg/kg) for MK-801 induced hyperactivity.

Cognitive impairment in schizophrenia is a core symptom (Elvevag and Goldberg, 2000). The antipsychotics currently in use have limited beneficial effects for cognitive impairment in schizophrenia. In the NORT, like clozapine (3.0 mg/kg, p.o.), T-251 (0.6 mg/kg, p.o.) significantly improved MK-801 induced cognitive deficit in rats. These results suggest that T-251 may have beneficial effect for cognitive impairment in patients with schizophrenia.

Finally, we investigated the side effect profile of T-251 in rats. EPS are the most major and serious adverse effects induced by current antipsychotics, leading to treatment withdrawal (Kane, 2001). Unlike haloperidol and olanzapine, T-251 and clozapine did not produce catalepsy at higher doses. In addition, T-251 did not increase plasma levels of prolactin 1 h after a single administration whereas haloperidol or olanzapine significantly increased them. Collectively, T-251 may be an antipsychotic with fewer side effects than current antipsychotics.

In this study, the efficacy of T-251 on negative symptoms of schizophrenia was not evaluated. Several other PDE10A inhibitors have been reported to be effective for negative symptoms in rodents (Grauer et al., 2009; Langen et al., 2012). These compounds are also reported to be effective for cognitive dysfunction in addition to positive symptoms and have fewer side effects compared to current antipsychotics. Thus, it is likely that T-251 may also be effective against negative symptoms, although further studies in animal models for negative symptoms are needed.

As described, clinical trials for PDE10A inhibitors for schizophrenia have so far been unsuccessful, but different compounds have shown different profiles for efficacy and side effects. The potential of PDE10A inhibitors for schizophrenia is still being explored; clinical trials for another PDE10A inhibitor, Lu AF11167, for the treatment of schizophrenia negative symptoms are now undergoing (clinicaltrials.gov, NCT03793712 and NCT03929497). T-251 is also anticipated to lead the way for PDE10A inhibitors as potential treatments for schizophrenia.

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Declaration of competing interest

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References

Arakawa, K., Maehara, S., Yuge, N., Ishikawa, M., Miyazaki, Y., Naba, H., Kato, Y., Nakao, K., 2016. Pharmacological characterization of a novel potent, selective, and orally active phosphodiesterase 10A inhibitor, PDM-042 [(E)-4-(2-(2-(5,8-dimethyl-[1,2,4]triazolo[1,5-a]pyrazin-2-yl)vinyl)-6-(pyrrolidin-1-yl)pyrimidin-4-yl)morpholine] in rats: potential for the treatment of schizophrenia. Pharmacol. Res. Perspect. 4 (4), e00241.

Arakawa, K., Nakao, K., Maehara, S., 2017. Dopamine D1 signaling involvement in the effects of the phosphodiesterase 10A inhibitor, PDM-042 on cognitive function and extrapyramidal side effect in rats. Behav. Brain Res. 317, 204–209.

Beaulieu, J.M., Espinoza, S., Gainetdinov, R.R., 2015. Dopamine receptors - IUPHAR review 13. Br. J. Pharmacol. 172 (1), 1–23.

Cadinu, D., Grayson, B., Podda, G., Harte, M.K., Doostdar, N., Neill, J.C., 2018. NMDA receptor antagonist rodent models for cognition in schizophrenia and identification of novel drug treatments, an update. Neuropharmacology 142, 41–62.

Citrome, L., 2014. Unmet needs in the treatment of schizophrenia: new targets to help different symptom domains. J. Clin. Psychiatry 75 (Suppl. 1), 21–26.

Corponi, F., Fabbri, C., Bitter, I., Montgomery, S., Vieta, E., Kasper, S., Pallanti, S., Serretti, A., 2019. Novel antipsychotics specificity profile: a clinically oriented review of lurasidone, brexpiprazole, cariprazine and lumateperone. Eur. Neuropsychopharmacol., 31255396. https://doi.org/10.1016/j.euroneuro.2019.06.008.

Correll, C.U., Citrome, L., Haddad, P.M., Lauriello, J., Olfson, M., Calloway, S.M., Kane, J.M., 2016. The use of long-acting injectable antipsychotics in schizophrenia: evaluating the evidence. J. Clin. Psychiatry 77 (Suppl. 3), 1–24.

De Hert, M., Detraux, J., Stubbs, B., 2016. Relationship between antipsychotic medication, serum prolactin levels and osteoporosis/osteoporotic fractures in patients with schizophrenia: a critical literature review. Expert Opin. Drug Saf. 15 (6), 809–823.

DeMartinis III, N., Lopez, R.N., Pickering, E.H., Schmidt, C.J., Gertsik, L., Walling, D.P., Ogden, A., 2019. A proof-of-concept study evaluating the phosphodiesterase 10A inhibitor PF-02545920 in the adjunctive treatment of suboptimally controlled symptoms of schizophrenia. J. Clin. Psychopharmacol. 39 (4), 318–328.

Elvevag, B., Goldberg, T.E., 2000. Cognitive impairment in schizophrenia is the core of the disorder. Crit. Rev. Neurobiol. 14 (1), 1–21.

Forray, C., Buller, R., 2017. Challenges and opportunities for the development of new antipsychotic drugs. Biochem. Pharmacol. 143, 10–24.

Fujishige, K., Kotera, J., Michibata, H., Yuasa, K., Takebayashi, S., Okumura, K., Omori, K., 1999a. Cloning and characterization of a novel human phosphodiesterase that hydrolyzes both cAMP and cGMP (PDE10A). J. Biol. Chem. 274 (26), 18438–18445.

Fujishige, K., Kotera, J., Omori, K., 1999b. Striatum- and testis-specific phosphodiesterase PDE10A isolation and characterization of a rat PDE10A. Eur. J. Biochem. 266 (3), 1118–1127.

Gamanuma, M., Yuasa, K., Sasaki, T., Sakurai, N., Kotera, J., Omori, K., 2003. Comparison of enzymatic characterization and gene organization of cyclic nucleotide phosphodiesterase 8 family in humans. Cell. Signal. 15 (6), 565–574.

Geerts, H., Spiros, A., Roberts, P., 2017. Phosphodiesterase 10 inhibitors in clinical development for CNS disorders. Expert. Rev. Neurother. 17 (6), 553–560.

Gobira, P.H., Ropke, J., Aguiar, D.C., Crippa, J.A., Moreira, F.A., 2013. Animal models for predicting the efficacy and side effects of antipsychotic drugs. Rev. Bras. Psiquiatr. 35 (Suppl. 2), S132–S139 (Sao Paulo, Brazil: 1999).

Grauer, S.M., Pulito, V.L., Navarra, R.L., Kelly, M.P., Kelley, C., Graf, R., Langen, B.,

Logue, S., Brennan, J., Jiang, L., Charych, E., Egerland, U., Liu, F., Marquis, K.L., Malamas, M., Hage, T., Comery, T.A., Brandon, N.J., 2009. Phosphodiesterase 10A inhibitor activity in preclinical models of the positive, cognitive, and negative symptoms of schizophrenia. J. Pharmacol. Exp. Ther. 331 (2), 574–590.

Guillena, S.R., Garcia de Diego, B.P., 2019. 68 cognitive impairment in schizophrenia. First-generation long-acting antipsychotics versus aripiprazole long-acting injectable. CNS Spectr. 24 (1), 211–212.

Gyertyan, I., Kiss, B., Saghy, K., Laszy, J., Szabo, G., Szabados, T., Gemesi, L.I., Pasztor, G., Zajer-Balazs, M., Kapas, M., Csongor, E.A., Domany, G., Tihanyi, K., Szombathelyi, Z., 2011. Cariprazine (RGH-188), a potent D3/D2 dopamine receptor partial agonist, binds to dopamine D3 receptors in vivo and shows antipsychotic-like and procognitive effects in rodents. Neurochem. Int. 59 (6), 925–935.

Kadoh, Y., Miyoshi, H., Matsumura, T., Tanaka, Y., Hongu, M., Kimura, M., Takedomi, K., Omori, K., Kotera, J., Sasaki, T., Kobayashi, T., Taniguchi, H., Watanabe, Y., Kojima, K., Sakamoto, T., Himiyama, T., Kawanishi, E., 2018. Discovery of 2-[(E)-2-(7-fluoro-3-methylquinoxalin-2-yl)vinyl]-6-pyrrolidin-1-yl-N-(tetrahydro-2H-pyran-4-yl)pyrimidin-4-amine hydrochloride as a highly selective PDE10A inhibitor. Chem. Pharm. Bull. (Tokyo) 66 (3), 243–250.

Kane, J.M., 2001. Extrapyramidal side effects are unacceptable. Eur. Neuropsychopharmacol. 11 (Suppl. 4), S397–S403.

Kehler, J., 2013. Phosphodiesterase 10A inhibitors: a 2009–2012 patent update. Expert Opin. Ther. Pat. 23 (1), 31–45.

Kehler, J., Nielsen, J., 2011. PDE10A inhibitors: novel therapeutic drugs for schizophrenia. Curr. Pharm. Des. 17 (2), 137–150.

Kotera, J., Fujishige, K., Yuasa, K., Omori, K., 1999. Characterization and phosphorylation of PDE10A2, a novel alternative splice variant of human phosphodiesterase that hydrolyzes cAMP and cGMP. Biochem. Biophys. Res. Commun. 261 (3), 551–557.

Kotera, J., Fujishige, K., Michibata, H., Yuasa, K., Kubo, A., Nakamura, Y., Omori, K., 2000. Characterization and effects of methyl-2-(4-aminophenyl)-1, 2-dihydro-1-oxo-7-(2-pyridinylmethoxy)-4-(3,4, 5-trimethoxyphenyl)-3-isoquinoline carboxylate sulfate (T-1032), a novel potent inhibitor of cGMP-binding cGMP-specific phosphodiesterase (PDE5). Biochem. Pharmacol. 60 (9), 1333–1341.

Krebs, M., Leopold, K., Hinzpeter, A., Schaefer, M., 2006. Current schizophrenia drugs: efficacy and side effects. Expert. Opin. Pharmacother. 7 (8), 1005–1016.

Langen, B., Dost, R., Egerland, U., Stange, H., Hoefgen, N., 2012. Effect of PDE10A inhibitors on MK-801-induced immobility in the forced swim test. Psychopharmacology 221 (2), 249–259.

Macek, T.A., McCue, M., Dong, X., Hanson, E., Goldsmith, P., Affinito, J., Mahableshwarkar, A.R., 2019. A phase 2, randomized, placebo-controlled study of the efficacy and safety of TAK-063 in subjects with an acute exacerbation of schizophrenia. Schizophr. Res. 204, 289–294.

Morimoto, T., Hashimoto, K., Yasumatsu, H., Tanaka, H., Fujimura, M., Kuriyama, M., Kimura, K., Takehara, S., Yamagami, K., 2002. Neuropharmacological profile of a novel potential atypical antipsychotic drug Y-931 (8-fluoro-12-(4-methylpiperazin-1-yl)-6H-[1]benzothieno[2,3-b][1,5] benzodiazepine maleate). Neuropsychopharmacology 26 (4), 456–467.

Sasaki, T., Kotera, J., Yuasa, K., Omori, K., 2000. Identification of human PDE7B, a cAMP-specific phosphodiesterase. Biochem. Biophys. Res. Commun. 271 (3), 575–583.

Seeger, T.F., Bartlett, B., Coskran, T.M., Culp, J.S., James, L.C., Krull, D.L., Lanfear, J., Ryan, A.M., Schmidt, C.J., Strick, C.A., Varghese, A.H., Williams, R.D., Wylie, P.G., Menniti, F.S., 2003. Immunohistochemical localization of PDE10A in the rat brain. Brain Res. 985 (2), 113–126.

Serretti, A., De Ronchi, D., Lorenzi, C., Berardi, D., 2004. New antipsychotics and schizophrenia: a review on efficacy and side effects. Curr. Med. Chem. 11 (3), 343–358.

Shiraishi, E., Suzuki, K., Harada, A., Suzuki, N., Kimura, H., 2016. The phosphodiesterase 10A selective inhibitor TAK-063 improves cognitive functions associated with schizophrenia in rodent models. J. Pharmacol. Exp. Ther. 356 (3), 587–595.

Snyder, G.L., Vanover, K.E., 2014. Intracellular signaling and approaches to the treatment of schizophrenia and associated cognitive impairment. Curr. Pharm. Des. 20 (31), 5093–5103.

Snyder, G.L., Vanover, K.E., 2017. PDE inhibitors for the treatment of schizophrenia. Adv. Neurobiol. 17, 385–409.

Soderling, S.H., Bayuga, S.J., Beavo, J.A., 1999. Isolation and characterization of a dual-substrate phosphodiesterase gene family: PDE10A. Proc. Natl. Acad. Sci. U. S. A. 96 (12), 7071–7076.

Suzuki, K., Kimura, H., 2018. TAK-063, a novel PDE10A inhibitor with balanced activation of direct and indirect pathways, provides a unique opportunity for the treatment of schizophrenia. CNS Neurosci. Ther. 24 (7), 604–614.

Suzuki, K., Harada, A., Shiraishi, E., Kimura, H., 2015. In vivo pharmacological characterization of TAK-063, a potent and selective phosphodiesterase 10A inhibitor with antipsychotic-like activity in rodents. J. Pharmacol. Exp. Ther. 352 (3), 471–479.

Suzuki, K., Harada, A., Suzuki, H., Miyamoto, M., Kimura, H., 2016. TAK-063, a PDE10A inhibitor with balanced activation of direct and indirect pathways, provides potent antipsychotic-like effects in multiple paradigms. Neuropsychopharmacology 41 (9), 2252–2262.

Thompson, W.J., Terasaki, W.L., Epstein, P.M., Strada, S.J., 1979. Assay of cyclic nucleotide phosphodiesterase and resolution of multiple molecular forms of the enzyme. Adv. Cyclic Nucleotide Res. 10, 69–92.

Tschoner, A., Engl, J., Laimer, M., Kaser, S., Rettenbacher, M., Fleischhacker, W.W., Patsch, J.R., Ebenbichler, C.F., 2007. Metabolic side effects of antipsychotic medication. Int. J. Clin. Pract. 61 (8), 1356–1370.

Wadenberg, M.L.G., 2010. Conditioned avoidance response in the development of new antipsychotics. Curr. Pharm. Des. 16 (3), 358–370.

Xie, Z., Adamowicz, W.O., Eldred, W.D., Jakowski, A.B., Kleiman, R.J., Morton, D.G., Stephenson, D.T., Strick, C.A., Williams, R.D., Menniti, F.S., 2006. Cellular and subcellular localization of PDE10A, a striatum-enriched phosphodiesterase. Neuroscience 139 (2), 597–607.

Yuasa, K., Kotera, J., Fujishige, K., Michibata, H., Sasaki, T., Omori, K., 2000. Isolation and characterization of two novel phosphodiesterase PDE11A variants showing unique structure and tissue-specific expression. J. Biol. Chem. 275 (40), 31469–31479.

Zagorska, A., Partyka, A., Bucki, A., Gawalskax, A., Czopek, A., Pawlowski, M., 2018. Phosphodiesterase 10 inhibitors - novel perspectives for psychiatric and neurodegenerative drug discovery. Curr. Med. Chem. 25 (29), 3455–3481.