1. TITLE PAGE

TPN672: A Novel Serotonin-Dopamine Activity Modulator for the Treatment of Schizophrenia

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Running Title: TPN672, A Novel Serotonin-Dopamine Activity Modulator

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**Non-Standard Abbreviations:**

- EPS: Extrapyramidal symptoms
- 5-HT: serotonin
- DA: Dopamine
- 5-HT$_{1A}$R: serotonin 1A receptor
- 5-HT$_{2A}$R: serotonin 2A receptor
- D$_2$R: Dopamine 2 receptor
- D$_3$R: Dopamine 3 receptor
- RPL: Prolactin
- RIS: Risperidone
- ARI: Aripiprazole
- PCP: Phencyclidine
- PFC: prefrontal cortex
- eEPSC: evoked excitatory postsynaptic current
- NOR: New object recognition

**Recommended section assignment for JPET:**

1) Drug Discovery,  
2) Neuropharmacology
3. Abstract

TPN672, 7-(2-(4-(benzothiophen-4-yl) piperazin-1-yl) ethyl) quinolin-2(1H)-one maleate, is a novel antipsychotic candidate with high affinity for serotonin and dopamine receptors, that is currently in clinical trial for the treatment of psychiatric disorders. In vitro binding study showed that TPN672 exhibited extremely high affinity for serotonin 1A receptor (5-HT$_{1A}$R) (Ki = 0.23 nM) and 5-HT$_{2A}$R (Ki = 2.58 nM) as well as moderate affinity for D$_3$ receptor (Ki = 11.55 nM) and D$_2$R (Ki = 17.91 nM). In vitro functional assays demonstrated that TPN672 acted as a potent 5-HT$_{1A}$R agonist, D$_2$R/D$_3$R partial agonist and 5-HT$_{2A}$R antagonist. TPN672 displayed robust antipsychotic efficacy, significantly higher than aripiprazole in rodent models (such as blocked phencyclidine-induced hyperactivity, and ameliorated negative symptoms and cognitive deficits in sociability test, dark avoidance response, Morris water maze test, and novel object recognition test). The results of electrophysiological experiments showed that TPN672 might inhibit the excitability of the glutamate system through activating 5-HT$_{1A}$R in mPFC, thereby improving cognitive and negative symptoms. Moreover, the safety margin (the ratio of minimum catalepsy inducing dose to minimum effective dose) of TPN672 was about 10-fold, superior to aripiprazole. In conclusion, TPN672 is a promising new drug candidate for the treatment of schizophrenia, and has been shown to be more effective in attenuating negative symptoms and cognitive deficits, meanwhile have lower risk of EPS (Extrapyramidal symptoms) and hyperprolactinemia.
4. Significance Statement

TPN672 is a promising new drug candidate for the treatment of schizophrenia and has been shown to be more effective in attenuating negative symptoms and cognitive deficits, meanwhile have a lower risk of EPS and hyperprolactinemia. A phase I clinical trial is now under way to test its tolerance, pharmacokinetics and pharmacodynamic effects in human volunteers. Accordingly, we believe that our present results will have significant impact on the development of new anti-schizophrenia drugs.
5. Introduction

Schizophrenia is a severe psychiatric disorder that has profoundly affects both the individuals affected and society. Schizophrenia is characterized by diverse psychopathology; the core features are positive symptoms (delusions and hallucinations; so-called psychotic symptoms in which contact with reality is lost), negative symptoms (impaired motivation, reduction in spontaneous speech, and social withdrawal), and cognitive impairment (temporary or permanent loss of mental function) (René S. Kahn et al. 2015; Owen et al. 2016; Krogmann et al. 2019). Schizophrenia is characterized by a sequential trajectory that involves four phases: premorbid phase, prodromal phase, psychotic phase and stable phase(Kelly et al. 2019). The psychotic phase is the formal onset of schizophrenia and is marked by repeated episodes of florid positive symptoms. Following the first psychotic break, psychotic symptoms become exacerbations or remissions across patients and through the course of the illness, and finally the disease reaches a stable phase, during which psychotic symptoms are less prominent and negative symptoms and the stable cognitive deficits increasingly predominate (Tandon et al. 2009). The negative and cognitive symptoms are associated with long-term effects on social function.

Antipsychotic drugs remain the main approach for the treatment of schizophrenia, but there are significant unmet medical needs in the current treatments. First, antipsychotic drugs may produce clinical benefits in psychotic patients through antagonism or partial agonism of the postsynaptic dopamine D2R. EPS are major side effects of first-generation antipsychotics, caused by the blockade of D2 receptors in the substantia nigra striatum, which is mainly manifested as abnormal movements, such as muscle spasms, rigidity, tremors, restlessness, and involuntary movements (such as tardive dyskinesia) [Casey DE, 1996]. Compared to first-generation antipsychotics, atypical antipsychotics that have antagonist activity on both D2R and 5-HT2A R
show fewer side effects related to EPS, but they also have other side effects such as dyslipidemia, weight gain, diabetes and prolonged QT interval, with no or limited effect on negative symptoms and cognitive dysfunction. Second, high levels of non-adherence, mainly because of side-effects such as movement disorders, metabolic and cardiometabolic side effects, cause poor recovery and relapse of symptoms (Galletly et al. 2016). Therefore, the discovery of new antipsychotics that are effective for negative and cognitive symptoms with reduced side-effects attracts great attention in both academia and industry.

Recent animal and clinical studies have shown that 5-HT\textsubscript{1A}R agonists can improve cognitive and negative symptoms of schizophrenia, and can reduce EPS caused by antipsychotic drugs. 5-HT\textsubscript{2A}R antagonists and D\textsubscript{3}R partial agonists can also reduce EPS caused by dopaminergic dysfunction (Meltzer et al. 2003; Kiss et al. 2008; Kiss et al. 2010).

The first 5-HTR subtype to be cloned and characterized by its high affinity for 5-HT was 5-HT\textsubscript{1A}R (Meltzer et al. 2003). The 5-HT\textsubscript{1A}R are expressed on the soma, presynaptic and postsynaptic membrane in hippocampal neurons. 5-HT\textsubscript{1A}R agonists can regulate 5-HT neuronal activity, thereby maintaining the balance of excitatory and inhibitory transmission. By enhancing the dopaminergic activity in the prefrontal cortex, 5-HT\textsubscript{1A}R agonism can improve the negative symptoms and cognitive impairment of patients (Meltzer et al. 2008; Celada et al. 2013), and can also improve the positive symptoms indirectly and alleviate EPS (Newman Tancredi 2010; Shimizu et al. 2010; Celada et al. 2013). 5-HT\textsubscript{1A}R can regulate GABAergic inhibitory interneurons by regulating glutamatergic excitatory neurons, exerting neuroprotective effects (Llado-Pelfort et al. 2012). Therefore 5-HT\textsubscript{1A}R may also be an important target for antipsychotics.
D₃R has been considered as a potential target for antipsychotic agents (Gross et al. 2012; Sun et al. 2016). D₃R are less abundant than their D₂ counterparts in the brain and are principally located in mesolimbic regions like the ventral striatum, islands of Calleja, nucleus accumbens, globus pallidus, and thalamus, but they are also found in the frontal and other cortical regions as well as the cerebellum (Watson et al. 2012). Mice genetically deficient in the D₃R show increased cognitive flexibility in the attentional set-shifting task and improved retention in a passive-avoidance test (Micale et al. 2010; Corponi et al. 2019). D₃R antagonists demonstrated procognitive effects in experimental learning paradigms (Millan et al. 2007; Gross et al. 2013) and inhibited haloperidol-induced catalepsy (Gyertyan and Saghy 2007). These findings suggested that D₃R antagonists might have some potential against negative symptoms and reduced EPS potential. Cariprazine, an antipsychotic drug with dopamine D₃R partial agonism, with better improvement of cognitive and negative symptoms effects in mice, has been successfully approved by the FDA (Kiss et al. 2010; Caccia et al. 2013). We hypothesized that a mixed D₃R/D₂R partial agonist could be an effective antipsychotic that would be free of EPS and have beneficial effects on cognition and negative symptoms (Kiss et al. 2010, Gyertyan et al. 2011).

Several atypical antipsychotic drugs, such as ziprasidone, lurasidone, aripiprazole, brexipiprazole, and cariprazine, have affinity for the 5-HT₁₅AR (5-HT₁₅AR), but their efficacy and side effects are different, presumably due to their balanced differences in 5-HT₁₅AR / 5-HT₂₅AR / D₂R / D₃R affinity. The best 5-HT₁₅AR / 5-HT₂₅AR / D₂R / D₃R affinity balance needs to be studied to improve the impact on cognitive dysfunction and reduce negative symptoms, EPS and metabolic side effects. Currently, the discovered atypical antipsychotic drugs have a lower affinity for 5-HT₁₅AR than D₂R and D₃R. Therefore, it may be of great significance to design and
evaluate compounds with higher affinity for 5-HT$_{1A}$R than D$_2$R, and comparable affinity for 5-HT$_{2A}$R and D$_3$R to D$_2$R.

Our study examined *in vitro* and *in vivo* activities of a novel antipsychotic candidate TPN672. TPN672(fig 1) (Patent Application: WO2019242717), 7-(2-(4-(benzothiophen-4-yl)piperazin-1-yl)ethyl) quinolin-2(1H)-one maleate, showed a powerful 5-HT$_{1A}$R agonistic effect, and has the unique characteristics of D$_2$R / D$_3$R partial agonistic and 5-HT$_{2A}$R antagonizing activity. Compared to other antipsychotics. TPN672 showed weaker EPS adverse effects, but significantly improved positive symptoms, negative symptoms, and cognitive impairments in phencyclidine (PCP) induced animal models of schizophrenia.

6. Materials and Methods

Animals

Male/Female ICR mice (20–25 g) and male SD rats (180–220 g) were used in this study. Experimental animals were housed in a thermostatically controlled room at 23±2° C and 55-65% relative humidity on 12h dark / light cycle (lights on from 6:00 to 18:00). All procedures on animals were approved by the Institutional Animal Care and Use Committee of Shanghai Institute of Materia Medica or China Pharmaceutical University.

Drugs

TPN672, brexipiprazole and PCP were synthesized by Shanghai Institute of Materia Medica, Chinese Academy of Sciences (CAS). Aripiprazole (ARI) and risperidone (RIS) were purchased from Jiangsu Nhwa Pharmaceutical Co. Ltd. WAY100635 were purchased from Sigma-Aldrich. Canine serum prolactin (PRL) ELISA kit was purchased from Abcam. All other chemicals are analytical level and obtained from commercial sources. In most experiments, TPN672 was used
as the maleate salt. In vivo experiments, PCP was dissolved in saline, injected intraperitoneally at a dose of 5 mg/kg of body weight for rats and 7 mg/kg for mice, while TPN672, aripiprazole, and risperidone were suspended with 0.5% CMC-Na, and administrated intragastrically at a volume of 10ml /kg body weight.

**Radioligand-receptor Binding Assays**

Human receptor binding assays were performed under the incubation conditions shown in table 1.

Radioligand-receptor binding assays were used to identify the affinity of TPN672, aripiprazole, risperidone and brexpiprazole for G protein-coupled receptors, and the protocols were performed according to previous reports with some modifications(Song et al. 2012). The reaction mixture containing 20-30 μg membrane receptor protein and 1-2nM [³H] labeled ligand was incubated at 30 °C for 50min, and then the reaction was stopped in ice-cold water. The reaction mixture was transferred to the millipore sample collector and filtered by GF / C (Whatman) glass fiber filter paper. The filter paper was rinsed with 50 mM Tris-HCl buffer (pH 7.4) and dried up. Radioactivity was quantified by Tri-Carb 2910TR (PerkinElmer LAS Ltd, Beaconsfield, UK) with a Lipophilic scintillation solution. IC₅₀ values were analyzed by nonlinear regression using Prism 8.0.2. Ki values were calculated according to Cheng-Prusoff equation: Ki=IC₅₀ / (1+S/Kd), where S is the radioligand concentration, and Kd is the dissociation constant (Ishibashi et al. 2010).

**Receptor functional activity**

Receptor functional activity were performed under the incubation conditions shown in table 2.
Ultra Lance cAMP assay was used to test 4 compounds on D1R, D4R, 5HT1B with antagonist mode and D2R, 5HT1A with agonist & antagonist mode. The compound was transferred to the assay plate by Echo, and then cells were collected with stimulation buffer. Ten microliter of the cell solution was transferred to assay plate, centrifuged at 600 rpm for 3 minutes, incubated for 60 minutes at room temperate, then 5 μl 4X Eu-cAMP tracer solution and 5 μl ULight-anti-cAMP solution were added to the assay plate, centrifuged at 600 rpm for 3 minutes and incubated for 60 minutes at room temperature. Finally the plate was read on EnVision. Readout parameters: Excitation 320 nm or 340 nm, Emission 615 nm/665 nm, Delay time: 20 μs and window time: 200 μs.

FLIPR calcium assay was used to test 4 compounds on H1, M1, Alpha1A, 5HT2A, 5HT2B, 5HT2C antagonist mode. The cells were inoculated in a 384-well plate and incubated for 16-24 hours in a 37°C and 5% CO2 incubator. Then the cells were taken out, the culture medium was removed, 30 μl calcium 5 dye was added to each well, and incubated for 1 hour at 37°C and 5% CO2. Compounds were transferred to the plate with 30 μl assay buffer by echo. Compounds were added 15 μl/well and incubated for 10 minutes at room temperature. Inducer was added 22.5 μl/well and the calcium flux signal was measured with FLIPR. Readout parameters: Excitation 470/495 nm, Emission 515 nm/575 nm, read interval: 1s, number of reads: 120 and number of reads before dispense: 10.

HTRF was used to evaluate the agonist and antagonist activity of human D3 receptor expressed in CHO cells. The cells were suspended in buffer (HBSS for agonist test; Hepes for antagonist test), and then distributed in the microplates with a density of 104 cells/well. Thereafter, NKH 477, an adenylate cyclase activator, was added at a final concentration of 1.5 μM. The reference agonist dopamine was added at a final concentration of 10 nM in the agonist
activity test. For basal control measurements of antagonist activity test, dopamine was omitted from the wells containing 1 µM butaclamol, a standard reference antagonist.). After incubated 30 min at 37°C, the cells were lysed and the fluorescent receptor (D2-labeled cAMP) and fluorescent donor (europium labeled anti-cAMP antibody) were added. After 60 min at room temperature, the fluorescence transfer was measured by a microplate reader (Envision, Perkin Elmer).

**Electrophysiology**

Concentric stimulation electrodes were placed on the second layer of the PrL brain area of the prefrontal cortex (PFC), and the whole-cell patch-clamp recording technique was used to record the AMPA receptor caused by the current stimulation on the pyramidal neurons in the fifth layer mediated eEPSC (evoked excitatory postsynaptic current). The changes of eEPSC were measured after perfusion of TPN672 (100 nM) with external fluid, and whether WAY-100635 can affect the effect of TPN672 on eEPSC were measured. Then double-pulse stimulation was used to further verify the effect of TPN672 on 5-HT1A-R-mediated changes in glutamate transmission.

**PCP-Induced Hyperactivity in Mice and Rats**

Locomotor activity in rats was measured using activity boxes (box for mice: 25 cm×25 cm×40 cm; box for rats: 45 cm×45 cm ×60 cm) equipped with video tracking software (EthoVision XT, Noldus). TPN672, Risperidone, aripiprazole, or vehicle was administered p.o. 1 hours before injection of PCP (7mg/kg s.c. for mice, 5 mg/kg s.c. for rats). The locomotor activity was measured immediately after PCP injection and recorded for 1 hour.

**Dark avoidance experiment**
The dark escape box (dimensions: 46×19.5×20 cm) was subdivided into two compartments by a hurdle (1 mm in width, 3 cm in height). There is a channel with a diameter of 5.0 cm between the two chambers and a copper grid at the bottom of the box.

Mice were injected subcutaneously with PCP 7.5 mg/kg or normal saline for 7 consecutive days, and 24 hours after the last PCP administration, the animals were given the corresponding dose of drug or vehicle by gavage for 5 consecutive days. The animals were trained 30 minutes after the last dose. Before training, the head of the mouse were put into the bright room with the back of the hole, adapted to the environment for 2 minutes, and then the 0.5mA was passed to the dark room copper grid. After entering the dark room, the mouse would escape to the bright room after being shocked. After 24 hours, the memory test of the mouse was performed. The time when the mouse entered the dark room for the first time (the dark avoidance incubation period) and the number of times the mouse entered the dark room (the number of errors) were recorded. If the mouse had not entered the dark room within 5 minutes, the incubation period was counted as 300s. The experimental observation indexes were the escape latency and the number of errors of mice.

Morris Water Maze

The Morris water maze consisted of a circular pool (120 cm diameter) that was filled with water (25±1 °C). The mice were trained in the Morris water maze over four daily sessions (S1, S2, S3, S4). An escape platform (9 cm in diameter ) was located in the center of the second quadrant 1 cm below the water surface. Black ink was added to the pool to reduce the visibility of the platform. Video tracking was conducted with a video camera focused on the full diameter of the pool. Navigation parameters were analyzed by using the video tracking system.
(EthoVision XT, Noldus). The four sessions were performed on consecutive days between 9:00 and 12:00.

The water maze experiment lasts 5 days, the first 4 days are the positioning navigation experiment (also the training phase), and the 5th day (that is, 24 hours after the end of the last training) was the space exploration experiment (also the test phase). After the positioning navigation experiment, the platform in the water labyrinth experimental device was removed, and the space exploration experiment was conducted 24 hours after the last training. For each daily trial, the mice were taken from the home cage and placed in one of the four randomly determined locations in the water maze with their heads facing the center of the water maze. A trial was started when the mouse was released from one of three randomly chosen start positions. After the rat found and climbed onto the platform, the trial was stopped and the escape latency was recorded. The maximum trial length was 90 s. In the test phase, a quadrant was selected randomly, the mice of each group were put into the water in order to make them swim freely for 90 second. Escape latency (s) of the mice to find the hidden platform were evaluated during each trial. If the mouse had not climbed onto the platform in 90 s, the experimenter guided the mouse to the platform by hand and recorded the escape latency of 90 s.

Mice were injected with PCP (7.5mg/kg) or saline intraperitoneally for 10 consecutive days. After the last injection of PCP, animals were given intragastrically the corresponding dose of drug or vehicle once a day for 7 consecutive days. The Morris water maze experiment started 24 hours after the last dose.

**Novel Object Recognition**
The procedure was modified according to Bevins (Bevins and Besheer 2006). The task was performed in a 40 cm×50 cm × 50 cm chamber. All animals were given a habituation session during which they were allowed to freely explore the environment for 10 min. No objects were placed in the chamber during the habituation trial. Twenty four hours after habituation, training was conducted by placing individual rats into a chamber for 3 min, in which two identical objects (object A1 and A2) were positioned in two adjacent corners, 10 cm away from the walls. In a short-term memory test performed 1 h after training, the mice explored the chamber for 3 min in the presence of one novel and one familiar object. Two triangular cones (3cm in bottom and 8cm in height) were used as familiar objects, and one cylinder (3cm in diameter and 8cm in height) was used as novel object. All objects presented had similar textures, colors, and sizes. The time animals spent on observing, licking, sniffing or touching the object was recorded as object interaction. Standing, sitting or leaning on the object is not recorded. The time spend on exploring new and old objects was recorded separately. SD rats were intraperitoneally injected with PCP (5 mg/kg) for 14 consecutive days, after which the animals were washed out for 3 days, and then were given the corresponding dose of drug or vehicle by gavage for 7 consecutive days (Day 18-Day 24) prior to the testing day. Object recognition in this test was reflected by discrimination index (( time with novel object - time with familiar object)/(time with novel object + time with familiar object )) (Jiyeon K. D, 2019).

Social Interaction Test

Social interaction test was performed in an open field box measured 40cm × 40cm × 50cm. Above the arena was a lamp (3.5 lx) which illuminated the open field. A video-camera was placed above the arena to record activity in the open-field. Each rat was acclimatised to the arena for a period of 10 min the day before testing. On the day of testing, rats were again paired with
unfamiliar weight-matched conspecifics which had received the same treatment and placed in the arena for 10 min. In this experiment, the time of rats spend on positive social behaviors such as combing their hairs, chasing each other's tails, crossing each other's body, and mutual licking was observed and recorded.

Rats were injected with PCP (5mg/kg) or saline intraperitoneally for 14 consecutive days. After the last injection of PCP, the animals were washed for 7 days, after which the animals were given the corresponding dose of drug or vehicle by gavage once a day for 7 consecutive days. The social experiment started 24 hours after the last dose.

Catalepsy Test

Male and Female ICR mice were used in this test. Animals were given intragastrically the corresponding dose of drug or vehicle. After 1, 2 and 4 hours of administration, the forepaws of mice were placed on a stainless-steel bar. If this position was kept for 20 s or longer, catalepsy would be judged to be positive.

Prolactin

Female ICR mice were single acute treatment with TPN672, risperidone and aripiprazole for the toxicity evaluation, blood was collected one hour after administered. Serum was prepared by centrifugation at 4 °C,1600g, and the prolactin concentration in the serum was detected with the mice serum prolactin ELISA kit.

Statistical analysis

In vitro assays, the concentration-response curves, Ki, IC₅₀, EC₅₀, and Eₘ₉ values were calculated by nonlinear regression analysis using GraphPad Prism 8.0.2. In vivo and electrophysiological experiments, data were analyzed using GraphPad Prism 8.0.2 and were
presented as means ± SEM. The data were analyzed statistically by one-way ANOVA and Dunnett’s post hoc test (A, B) or two-way ANOVA and Dunnett’s post hoc test. Student’s t-test (for comparisons between two groups) was used in the social interaction test between vehicle-treated and model group and the electrophysiological experiments. $P < 0.05$ was set as statistical significance.

7. Results

**In Vitro Pharmacology**

To determine the molecular targets that mediate the response to TPN672, the compounds were tested against several known molecular targets (G protein-coupled receptors). Table 2 summarizes the affinity values, and of particular interest are the relatively high affinities of TPN672 for 5HT$_{1A}$ (Ki=0.23 nM), which is 1624.3-and 30.2-folds to that of risperidone and aripiprazole, respectively, and it had higher selectivity for 5HT$_{1A}$ related to D$_2$ with a ratio of 77.8 (determined by the affinity value of 5-HT$_{1A}$R divided by that of D$_2$R) than risperidone (ratio<0.01), aripiprazole (ratio=0.59), brexipiprazole (ratio=2.5) and cariprazine (ratio=0.23) (Table 3). It is also interesting that TPN672 showed a good balance between D$_2$ and D$_3$R, whose Ki values are 17.91 and 11.55 nM, respectively. The follow-up functional profile testing showed that TPN672 exhibited a broad range of activities at several receptors including D$_2$, D$_3$, 5-HT$_{1A}$ and 5-HT$_{2A}$R (Table 4, Fig 2.). The most notable activity of TPN672 was its agonistic activity to 5-HT$_{1A}$R (maximum efficacy=100%, EC$_{50}$=0.28 nM), which is far more potent than that of aripiprazole (EC$_{50}$=159 nM) and brexipiprazole (EC$_{50}$=66.8 nM). The maximum agonistic and antagonistic activities of TPN672 for D$_2$R were 27.52% and 61.71%, respectively; for D$_3$R were 59.0% and 19.3%, respectively. Its maximal antagonistic activity for 5-HT$_{2A}$R was 99.6%.
Electrophysiology

We performed whole-cell patch clamp recording in brain slice containing the PrL and used concentric bipolar electrode to stimulate fibers containing glutamatergic afferents to the PrL (Fig 3a). EPSCs were evoked by local electrical stimulation in the presence of GABA<sub>A</sub> receptor antagonist PTX and NMDA receptor antagonist D-APV. As illustrated in Figure 3b and 3c, the tested sample PrL neuron was clamped at -70 mV, and the recorded eEPSC were totally blocked by AMPA receptor antagonist NBQX, indicating that the recorded eEPSCs are mediated by AMPA receptor. Then we examined the effect of TPN672 on eEPSCs in PrL to address whether it could modulate glutamate transmission. The results showed that TPN672 (10 μM) induced a significant decrease in the amplitude of eEPSCs to 67.51 ± 3.97% of the control (n = 10, P < 0.001, paired t test) and this inhibitory effect of TPN672 on glutamatergic eEPSCs could be washed out (fig 3d,e). To confirm whether the decreased glutamatergic transmission via a presynaptic mechanism, we further examined the effect of TPN672 on the paired pulse ratio (PPR). As shown in fig 3.f, stimuli elicited a pair of eEPSCs with the second eEPSC amplitude larger than the first one. Application of TPN672 reduced the peak amplitude of both EPSCs. However, TPN672 significantly enhanced the paired pulse facilitation of eEPSCs from 1.13 ± 0.15 to 1.35 ± 0.15 (n = 5, p < 0.05, paired t test; fig 3.g), strongly suggesting that a presynaptic mechanism mediates the inhibition of eEPSCs induced by TPN672 in PFC. Finally, bath application of WAY100635 (3 μM), a 5-HT<sub>1A</sub>R receptor antagonist, significantly blocked the inhibition of TPN672 on eEPSCs (n > 5, p > 0.05, paired t test, Fig 3. h and Fig 3. i).

Effects of TPN672 on PCP-Induced Hyperactivity in Mice and Rats

Acute treatment of PCP can induce robust hyperactivity in rodents, similar to the positive symptoms of schizophrenic patients, and is used as a routine assay in preclinical studies and is
widely used to screen novel compounds with antipsychotic effects (Moffat, Vincent et al. 2017, Dedic, Jones et al. 2019).

In the open field test in mice, results of the two-way ANOVA showed that drugs treatment was the only significant factor ($p < 0.001$, F(6,124)=8.41), while there were no significant differences among sex ($p=0.7327$, F(1,124)=0.1171). TPN672 significantly inhibited the increase in spontaneous movement distance of animals induced by PCP in a dose-dependent manner (Fig 4). Mice received 0.1-0.3 mg/kg TPN672 (0.1 and 0.3 mg/kg, $p=0.0140$ and $p=0.0001$, respectively) showed effects similar to animals received 1 mg/kg aripiprazole ($p=0.0134$) or 0.1 mg/kg risperidone ($p=0.0001$) (two-way ANOVA and Dunnett’s post hoc test). Rats administered 3-10 mg/kg TPN672 showed similar or better effects than animals administered 10 mg/kg aripiprazole (3 and 10 mg/kg TPN672, $p=0.0037$ and $p < 0.0001$, respectively; 10 mg/kg aripiprazole, $p=0.0179$)(one-way ANOVA test with Dunnett’s post hoc test).

**Effect of TPN672 on social interaction behavior of model rats**

PCP at a dose of 5 mg/kg significantly decreased the total time spent in social interaction ($p=0.0489$ vs. vehicle). TPN672 improved the social deficits caused by PCP (Fig 5.). Post-hoc analysis of one-way analysis variance for multiple comparisons showed that administration of TPN672 1mg/kg significantly improved the social deficits in PCP rat model compared with untreated group ($p=0.0305$). The positive control, aripiprazole, at doses of 3 mg/kg also significantly improved the social deficits caused by PCP ($p=0.042$) (Fig 5).

**Effects of TPN672 on cognitive deficits**
To examine potential therapeutic effects of TPN672 on cognitive deficits commonly associated with schizophrenia, we performed three experiments. In passive avoidance task, we observed that PCP reduced passive avoidance (PA) error latency \((p<0.0001\) vs. vehicle) and drastically reduced times of PA error \((p=0.0002\) vs. vehicle), suggesting impaired cognition. TPN672 (0.025 and 0.5 mg/kg) induced a dose-dependent inhibition of PA error latency \((p=0.0129\) and \(p<0.0001\), respectively) and PA error times \((p=0.0022\) and \(p<0.0001\), respectively), similar to aripiprazole (0.4 mg/kg, \(p<0.0001\) for error latency and \(p<0.0001\) for times of PA error) (Fig 6.a and b).

Morris water maze test has been widely used to examine spatial learning and memory in rodents. Results of the two-way ANOVA showed that drugs treatment was the only significant factor \((p=0.0002, F(5,93)=5.329)\), while there were no significant differences among sex \((p=0.9419, F=(1,93)=0.0053)\). The latency to locate the platform was obviously reduced by both 0.05 and 0.1 mg/kg TPN672 treated mice in the test phase (Day 4) of the Morris water maze \((p=0.0001\) and \(p=0.0003\), respectively). TPN672 showed comparable effects at 0.05 and 0.1mg/kg to that of aripiprazole treatment at 0.4 mg/kg. The behavioral data demonstrated that TPN672 effectively alleviated PCP-induced impairments in spatial learning and memory (0.05-0.1 mg/kg, p.o., Fig 6.c).

Novel object recognition was used to directly examine the effects of TPN672 on cognitive deficits in PCP treated rats. In the 3-minute test experiment, rats treated with vehicle showed significant NOR damage by spending approximately equal time exploring a familiar object and a novel object. We found that TPN672 (0.3 mg/kg, p.o.) exhibited comparable effects to that of aripiprazole (3 mg/kg, p.o.) (Fig 6.d). Both treatments increased the discrimination index (3
mg/kg ARI, \( p = 0.0133 \); 0.3 mg/kg TPN672, \( p = 0.0079 \), one indicator of recovered cognitive functions.

**Adverse reactions related to D\(_2\)R antagonism**

**Catalepsy**: The minimum dose of TPN672 that induced catalepsy in mice is 1 mg/kg. The dose ratios of cataleptic activity against the inhibitory effect on dopaminergic stimulant-induced behavior and PA tests were 10 for TPN672 and 1 for aripiprazole and risperidone (Table 5).

**Hyperprolactinemia**: D\(_2\)R antagonism of schizophrenic drugs has the risk of inducing hyperprolactinemia in animals. Mice were given TPN672, risperidone and aripiprazole orally for single dose. We found that TPN672 (0.3 mg/kg, p.o.) and aripiprazole (3 mg/kg, p.o.) shown no significant difference in serum prolactin level compared with the vehicle-treated mice (Fig 7), while risperidone (0.1 mg/kg) induced a significant increase \( (p = 0.0099 \text{ vs. vehicle}) \) (Fig 7).

**8. Discussion**

Our results demonstrate that TPN672 is a novel antipsychotic drug with unique characteristics of 5-HT\(_{1\text{A}}\)R agonism, D\(_2\)R/D\(_3\)R partial agonism and 5-HT\(_{2\text{A}}\)R antagonism.

Common adverse events of schizophrenia drugs include headache, insomnia and restlessness, hyperprolactinemia and weight gain. Preclinical and clinical studies suggested that the insufficient signal transduction of D\(_1\)R in prefrontal lobe results in cognitive impairment [Goldman-Rakic, 2004]. The affinity of TPN672 for D\(_1\)R was roughly equivalent to D\(_2\)R and D\(_3\)R, but the antagonistic activity for D\(_2\)R and D\(_3\)R was 26-fold and 17-fold for D\(_1\)R, which suggested that TPN672 had weak D\(_1\)R antagonistic activity, and may have low propensity for causing cognitive impairment. *In vitro* functional activity assays, TPN672 had week antagonistic activity on D\(_4\) receptor (related to the positive symptoms of schizophrenia), D\(_1\) receptor (related to
cognitive impairment), $\alpha_{1A}$ receptor (related to orthostatic hypotension), $H_1$ receptor (related to sedation and weight gain) or cholinergic $M_1$ receptor (related to cognitive impairment) (Table 3) [Elizabeth, 2008; Kroeze, 2003; Saibal, 2016]. In vivo animal experiments results indicate a low risk of EPS and hyperprolactinemia after TPN672 treatment. Research results indicate that TPN672 is expected to be safety and tolerable in clinical practice.

The results of efficacy evaluation suggest that TPN672 not only treats positive symptoms of schizophrenia but also improves negative symptoms and cognitive impairments in the PCP-induced schizophrenia model.

Distinct from other antipsychotics, TPN672 in vitro exhibits powerful affinity to 5-HT$_{1A}$R, and similar affinities to $D_3$R ($K_i=11.55$ nM) and $D_2$R ($K_i=17.91$ nM) (Table 2).

In addition, TPN672 dose-dependently reduced the PCP-induced hyperactivity in mice and rats, suggesting the potential efficacy of TPN672 on the positive symptoms in schizophrenia. The antagonistic activities of TPN672 on $D_2$R and $D_3$R can inhibit the over-activation of dopaminergic neurons. Moreover, 5-HT$_{1A}$R agonistic activity can reduce glutamate release, thereby decreasing dopamine release in the midbrain and ameliorating the positive symptoms of patients.

At present, negative symptoms and cognitive disorders of schizophrenia cannot be effectively managed, that hinders schizophrenic patients from returning to normal life$^{31,32}$. Sub-chronic administration of PCP is a widely employed animal models to replicate negative symptoms and cognitive impairments of schizophrenic patients in antipsychotic research. We used this approach to induce schizophrenia-like behaviors in rodents and performed a sociability test to assess the effects of TPN672 on negative symptoms. Meanwhile, we used the passive avoidance task, the water maze experiment, and the novel object recognition experiment to
evaluate the reversal of cognitive impairment by TPN672. Our results show that TPN672 significantly improved negative symptoms. In the passive avoidance task, the water maze task and the novel object recognition test, TPN672 treatment was effective on cognition improvement.

According to the results of the EPS evaluation experiment, the safety margin of TPN672 is 10 times that of risperidone and aripiprazole, which may translate into a better clinical safety/compliance profile. 5-HT\textsubscript{1A}R and 5-HT\textsubscript{2A}R in the prefrontal cortex are mostly expressed in pyramidal neurons. 5-HT\textsubscript{1A}R agonism and/or 5-HT\textsubscript{2A}R antagonism may indirectly regulate the balance of excitatory and inhibitory transmission in pyramidal neurons. Studies have found that selective 5-HT\textsubscript{1A}R agonists can stimulate the release of nigrostriatal DA and reverse the extrapyramidal side effects (EPS) caused by D\textsubscript{2}R antagonists (Nunez 2008; Shimizu et al. 2010).

The imbalance in striatal dopamine levels can lead to a disorder of prolactin secretion (Vanover et al. 2018). Most anti-schizophrenia drugs, such as risperidone, have side effects that induce hyperprolactinemia, which can lead to sexual dysfunction or aggravation of negative symptoms (Jolene R. Bostwick 2009; Besnard et al. 2014; Peuskens et al. 2014). Therefore, prolactin levels were measured in the safety evaluation following single dosing of TPN672. TPN672 had no effect on the prolactin levels at a dose of 0.3 mg/kg in mice, which is 3-fold of the minimum effective dose (0.1 mg/kg) in mice, suggesting a low risk of hyperprolactinemia.

The glutamate metabolism pathway is a potential molecular mechanism that affects schizophrenia cognition (Thomas et al. 2017). AMPAR is one of the family of glutamate receptors and is considered a suitable target for drug discovery and development because they play a key role in synaptic plasticity, which is the foundation of learning and memory, including long-term potentiation (LTP) and long-term depression (LTD) (Partin 2015). AMPAR were implicated in the cellular mechanisms underlying the observed antidepressant effects of
subanesthetic doses of ketamine (Anil K. Malhotra et al. 1996). The results of electrophysiological experiments (Fig 3.) showed that TPN672 may inhibit the glutamatergic transmission mediated by AMPAR between synapses and its mechanism may be related to the activation of presynaptic 5-HT1AR. TPN672 may inhibit the excitability of the glutamatergic system through the 5-HT1AR, thereby improving cognitive and negative symptoms.

Postmortem and neuroimaging studies have shown that the 5-HT1AR density in the cortex and amygdala of patients with schizophrenia has changed. Evidence in rodent models suggests that activation of 5-HT1AR can block D2R-induced EPS, regulate dopaminergic neurotransmission in the frontal cortex, have a positive effect on mood, and ameliorate NMDAR antagonists-induced cognitive and social interaction disorders (Celada et al. 2013). A new drug candidate for schizophrenia treatment, SEP-363856, was reported to be a trace amine-related receptor 1 (TAAR1) and 5-HT1AR agonist (Dedic et al. 2019; Koblan et al. 2020). Interestingly, early research shows that the selective agonist of TAAR1, RO5166017, can increase the efficacy of 5-HT1AR partial agonists and change the desensitization rate of 5-HT1A auto-receptor in DRN (Revel et al. 2011). Although SEP-363856 does not have D2R occupancy, it still exhibits antipsychotic-like behavioral characteristics, indicating that anti-schizophrenic drugs do not necessarily have D2R antagonistic effects. This further suggests the important role of 5-HT1AR agonistic activity in the development of anti-schizophrenic drugs.

TPN672 is a novel anti-schizophrenic drug with 5-HT1AR > 5-HT2AR > D2R ≈ D3R activity characteristics, showing robust efficacy in PCP-induced positive, negative and cognitive impairment animal models. Its strong activation of 5-HT1AR may contribute to higher efficacy and safety profile and overcome the limitations of current antipsychotic drugs, and reduce the risk of recurrence. Partial agonism of D2R and agonism of 5-HT1AR combined may lower the risk of
side effects such as EPS and hyperprolactinemia synergistically, and are expected to increase patient compliance. TPN672 is currently being investigated in phase I clinical trial in China (ClinicalTrials.gov Identifier: NCT03931668).
9. ACKNOWLEDGEMENTS

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10. AUTHOR CONTRIBUTIONS

Participated in research design: Ling He, Zhen Wang, Jingshan Shen, and Yu Wang.

Conducted experiments: Linyin Feng, Yanmin Peng, Chunhui Wu, Yu Wang.

Contributed new reagents or analytic tools: Yu Wang, Feipu Yang and Melkamu Alemu Abame.

Performed data analysis: Ling He, Yu Wang, Zhen Wang and Yang He.

Wrote or contributed to the writing of the manuscript: Yu Wang, Ling He and Zhen Wang.

No author has an actual or perceived conflict of interest with the contents of this article.
11. References


Celada, P., A. Bortolozzi and F. Artigas (2013). Serotonin 5-HT1A receptors as targets for agents to treat psychiatric disorders: rationale and current status of research. CNS Drugs 27(9): 703-716.


administration in rats and mice, but not in D3 receptor-knockout mice. Addict Biol 17(2): 259-273.


12. FOOTNOTES

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13. FIGURE LEGENDS

Fig 1. Chemical structure of TPN672

Fig 2. Effects of TPN672 on D2R, D3R, 5-HT1AR and 5-HT2AR, compared with antipsychotic drugs aripiprazole, risperidone and brexpiprazole. The effects are determined by cAMP production or intracellular Ca2+ concentrations. The concentrations of test compounds range from 0.03 nM to 100 μM. Data are presented as mean ± SEM (n=3) and show percentage rate of baseline.

Fig 3. Activation of 5-HT1AR inhibits the glutamatergic transmission in the mPFC pyramidal neurons. a. The image shows the placement of stimulating electrode and the recorded mPFC neuron on a coronal brain slice. b-c Raw traces show the APMA receptor-mediated eEPSC on mPFC neuron in the presence of PTX. Note that the fast evoked EPSC was totally blocked in the presence of AMPA/KA receptor antagonist NBQX. d. Bath application of TPN672, a highly potent artificial synthetic 5-HT1AR agonist, decrease the amplitude of eEPSCs, and this effect could be washed out. e. Bar graphs show the effect of TPN672 on the AMPA-mediated eEPSC. f. TPN672-induced decrease in amplitude of the eEPSCs was associated with an increase in the PPR. g. The plots and group data of the mean PPR obtained in the absence and presence of TPN672. h-i. The inhibitory effect of TPN672 on eEPSCs was blocked by selective 5HT1AR antagonist WAY100635 (n = 5). *p < 0.05 and ***p < 0.001 vs. control, Student’s t-test.

Fig 4. Single acute treatment with TPN672, ARI, and RIS inhibited the hyperactivity induced by PCP. a Inhibition of hyperactivity in rats (n=12-13 males per group); b Inhibition of
hyperactivity in mice (n = pooled analysis of 10 males and 9-10 females per group). Data are presented as mean ± SEM; ### $p < 0.001$ vs. vehicle, two-way ANOVA and Dunnett’s post hoc test; *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ vs. vehicle +PCP, one-way ANOVA test with Dunnett’s post hoc test.

**Fig 5. Effects of TPN672 and aripiprazole on PCP-induced social deficits in rats.** (n=12-14 males per group). Data are presented as mean ± SEM; $p < 0.05$ vs. vehicle, Student’s t-test; *$p < 0.05$ vs. vehicle +PCP, one-way ANOVA test with Dunnett’s post hoc test.

**Fig 6.** Effects of TPN672 on cognitive deficits induced by PCP. a and b Passive avoidance experiment (N=20 females per group), one-way ANOVA test with Dunnett’s post hoc test; c. Morris water maze test (N=10 males and 10 females per group), two-way ANOVA and Dunnett’s post hoc test; d. Novel object recognition test (N=8-12 males per group), one-way ANOVA test with Dunnett’s post hoc test. Data are presented as mean ± SEM; $p < 0.05$, ###$p < 0.001$ vs. vehicle, one-way ANOVA test with Dunnett’s post hoc test; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs. vehicle +PCP.

**Fig 7.** Effect of acute administration of TPN672, ARI and RIS on the serum prolactin content of female mice. N = 5 per group. Data are presented as mean ± SEM; *$p < 0.05$ vs. vehicle, one-way ANOVA test with Dunnett’s post hoc test.
14. TABLES

Table 1. Summary of binding assay conditions for human receptor assay in vitro

<table>
<thead>
<tr>
<th>Receptors</th>
<th>Source</th>
<th>Radioligand</th>
<th>Cold Ligand</th>
<th>Incubation</th>
<th>Incubation Time and Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>D&lt;sub&gt;1&lt;/sub&gt;</td>
<td>HEK293</td>
<td>[&lt;sup&gt;3&lt;/sup&gt;H]SCH23390(0.4)</td>
<td>Butaclamol</td>
<td>50 mM Tris-HCl</td>
<td>50 minutes, 30°C (PH 7.4)</td>
</tr>
<tr>
<td>D&lt;sub&gt;2&lt;/sub&gt;</td>
<td>HEK293</td>
<td>[&lt;sup&gt;3&lt;/sup&gt;H]Spiperone(0.06)</td>
<td>Butaclamol</td>
<td>50 mM Tris-HCl</td>
<td>50 minutes, 30°C (PH 7.4)</td>
</tr>
<tr>
<td>D&lt;sub&gt;3&lt;/sub&gt;</td>
<td>HEK293</td>
<td>[&lt;sup&gt;3&lt;/sup&gt;H]Spiperone(0.5)</td>
<td>Butaclamol</td>
<td>50 mM Tris-HCl</td>
<td>50 minutes, 30°C (PH 7.4)</td>
</tr>
<tr>
<td>5-HT&lt;sub&gt;1A&lt;/sub&gt;</td>
<td>HEK293</td>
<td>[&lt;sup&gt;3&lt;/sup&gt;H]8-OH-DPAT(1.745)</td>
<td>5-HT</td>
<td>50 mM Tris-HCl</td>
<td>50 minutes, 30°C (PH 7.4)</td>
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<tr>
<td>5-HT&lt;sub&gt;2A&lt;/sub&gt;</td>
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<td>Butaclamol</td>
<td>50 Tris-HCl (PH 7.4)</td>
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Table 2. Summary of receptor function activity test conditions in vitro

<table>
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<tr>
<th>Target</th>
<th>Cell line</th>
<th>Cell density</th>
<th>Coupled Mode</th>
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<tr>
<td>D₁ receptor</td>
<td>HEK293</td>
<td>2000 Cell/well</td>
<td>Gs</td>
</tr>
<tr>
<td>D₂ receptor</td>
<td>HEK293</td>
<td>2500 Cell/well</td>
<td>Gi</td>
</tr>
<tr>
<td>D₄ receptor</td>
<td>CHO-K1</td>
<td>10000 Cell/well</td>
<td>Gi</td>
</tr>
<tr>
<td>5HT₁ₐ receptor</td>
<td>HEK293</td>
<td>1000 Cell/well</td>
<td>Gi</td>
</tr>
<tr>
<td>5HT₁₈ receptor</td>
<td>HEK293</td>
<td>2000 Cell/well</td>
<td>Gi</td>
</tr>
<tr>
<td>H₁ receptor</td>
<td>CHO/K1</td>
<td>20000 Cell/well</td>
<td>Gq</td>
</tr>
<tr>
<td>M₁ receptor</td>
<td>CHO/K1</td>
<td>10000 Cell/well</td>
<td>Gq</td>
</tr>
<tr>
<td>Alpha₁A receptor</td>
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<td>Gq</td>
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<td>5HT₂ₐ receptor</td>
<td>CHO/K1</td>
<td>10000 Cell/well</td>
<td>Gq</td>
</tr>
<tr>
<td>5HT₂₈ receptor</td>
<td>CHO/K1</td>
<td>10000 Cell/well</td>
<td>Gq</td>
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</table>
Table 3. Binding affinities for cloned human receptor in vitro (Mean±SEM, n=3)

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Risperidone</th>
<th>Aripiprazole</th>
<th>Brexpiprazole</th>
<th>TPN672</th>
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</thead>
<tbody>
<tr>
<td>D₁</td>
<td>40.26±1.24</td>
<td>835.39±50.18</td>
<td>NT</td>
<td>22.56±0.96</td>
</tr>
<tr>
<td>D₂</td>
<td>2.30±0.19</td>
<td>4.09±0.43</td>
<td>0.30&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.91±0.31</td>
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<tr>
<td>D₃</td>
<td>43.01±0.56</td>
<td>52.16±1.85</td>
<td>1.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.55±1.74</td>
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<tr>
<td>5-HT&lt;sub&gt;1A&lt;/sub&gt;</td>
<td>373.59±44.23</td>
<td>6.94±0.81</td>
<td>0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.23±0.01</td>
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<tr>
<td>5-HT&lt;sub&gt;2A&lt;/sub&gt;</td>
<td>0.22±0.02</td>
<td>19.20±2.10</td>
<td>0.47&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.58±0.28</td>
</tr>
</tbody>
</table>

Data represent Ki obtained from three experiments performed in duplicate for each concentration and expressed as mean values (nonlinear regression analysis).

N.T., not tested; <sup>a</sup>Data from Kenji Maeda et al., 2014.
Table 4. Functional effects of TPN672 on human receptor in vitro (IC$_{50}$/EC$_{50}$, nM)

<table>
<thead>
<tr>
<th>Compounds</th>
<th>D$_2$</th>
<th>D$_3$</th>
<th>5-HT$_{1A}$</th>
<th>D$_1$</th>
<th>D$_2$</th>
<th>D$_3$</th>
<th>D$_4$</th>
<th>5-HT$_{1A}$</th>
<th>H$_1$</th>
<th>M$_1$</th>
<th>$\alpha_{1A}$</th>
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<tr>
<td></td>
<td>$E_{\text{max}}$</td>
<td>EC$_{50}$</td>
<td>$E_{\text{max}}$</td>
<td>EC$_{50}$</td>
<td>$E_{\text{max}}$</td>
<td>EC$_{50}$</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>TPN672</td>
<td>27.52</td>
<td>0.42</td>
<td>57.71</td>
<td>1.60</td>
<td>100</td>
<td>0.28</td>
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</tr>
<tr>
<td>Aripiprazole</td>
<td>30.66</td>
<td>8.05</td>
<td>N.T.</td>
<td>N.T.</td>
<td>87.68</td>
<td>159</td>
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<tr>
<td>Risperidone</td>
<td>-2.28</td>
<td>&gt;10,000</td>
<td>N.T.</td>
<td>N.T.</td>
<td>19.86</td>
<td>&gt;10,000</td>
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<tr>
<td>Brexpiprazole</td>
<td>23.2</td>
<td>17.4</td>
<td>N.S.</td>
<td>N.S.</td>
<td>69.25</td>
<td>66.8</td>
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<tr>
<td>Cariprazine</td>
<td>N.S.$^a$</td>
<td>N.S.$^a$</td>
<td>70</td>
<td>9.6</td>
<td>38.6$^a$</td>
<td>49.2$^a$</td>
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</table>

Data represent IC$_{50}$ or EC$_{50}$ (nonlinear regression analysis) obtained from a single experiment performed in triplicate for each concentration.

N.S., no stimulation; N.T., not tested; $^a$Data from Bela Kiss, et al., 2010.
Table 5. EPS liability of TPN672 and other antipsychotics

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Minimum dose inducing EPS (mg/kg)\textsuperscript{a}</th>
<th>Minimum effective dose (mg/kg)</th>
<th>Ratio\textsuperscript{b}</th>
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<tr>
<td>Aripiprazole</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Risperidone</td>
<td>0.1</td>
<td>0.1</td>
<td>1</td>
</tr>
<tr>
<td>TPN672</td>
<td>1</td>
<td>0.1</td>
<td>10</td>
</tr>
</tbody>
</table>

\textsuperscript{a} n=10 mice / sex / group;

\textsuperscript{b} Ratio is the ratio of the lowest dose of TPN672 that produces EPS to the lowest dose of its ant-dopaminergic activity (the lowest dose that produces inhibitory activity against PCP-induced high spontaneous activity in mice)
Figure 1.
Figure 2.

(a) D₂R agonist
- TPN672
- Aripiprazole
- Risperidone
- Brexpiprazole

(b) D₂R antagonist
- TPN672
- Aripiprazole
- Risperidone
- Brexpiprazole

(c) D₃R agonist
- TPN672
- Brexpiprazole
- Cariprazine

(d) D₃R antagonist
- TPN672

(e) 5-HT₁AR agonist
- TPN672
- Aripiprazole
- Risperidone
- Brexpiprazole

(f) 5-HT₂AR antagonist
- TPN672
- Aripiprazole
- Risperidone
- Brexpiprazole
Figure 5.
Figure 6.

(a) Error latency (sec) vs. dose of ARI and TPN672 with PCP. 
(b) Error Times vs. dose of ARI and TPN672 with PCP. 
(c) Escape latency (s) vs. time (days) with different treatments. 
(d) Discrimination index vs. dose of ARI and TPN672 with PCP.
Figure 7.

![Graph showing PRL levels with mg/kg of Veh, RIS(0.1), ARI(3), and TPN672(0.3).](image-url)