J Pharm Biopharm Res, 2022, 3(2): 228-236
DOI: 10.25082/JPBR.2021.02.003

RESEARCH ARTICLE

Pharmacodynamics study of a new 5-HT$_{2A}$ receptor inverse agonist PCC03039

Sujie Liu[1] **Xin Wang**[1] **Huan Gao**[1] **Liang Ye**[2] **Jingwei Tian**[1,2] **Guangying Du**[1*]

[1] School of Pharmacy, Collaborative Innovation Center of Advanced Drug Delivery System and Biotech Drugs in Universities of Shangdong, Key Laboratory of Molecular Pharmacology and Drug Evaluation (Yantai University), Ministry of Education, Yantai University, Yantai 264005, China

[2] R & D Center, Luye Pharma Group Ltd., Yantai 264003, China

Correspondence to: Guangying Du, School of Pharmacy, Collaborative Innovation Center of Advanced Drug Delivery System and Biotech Drugs in Universities of Shangdong, Key Laboratory of Molecular Pharmacology and Drug Evaluation (Yantai University), Ministry of Education, Yantai University, Yantai 264005, China; Email: duguangying@126.com

Received: March 1, 2022;
Accepted: March 17, 2022;
Published: March 21, 2022.

Citation: Liu S, Wang X, Gao H, *et al*. Pharmacodynamics study of a new 5-HT$_{2A}$ receptor inverse agonist PCC03039. *J Pharm Biopharm Res*, 2022, **3**(2): 228-236. https://doi.org/10.25082/JPBR.2021.02.003

Abstract: The purpose of this paper is to evaluate the pharmacodynamics of a new 5-HT$_{2A}$ receptor inverse agonist PCC03039 and provide data support for its druggability and clinical trial application. In the *in vitro* efficacy studies, the affinities of PCC03039 for 5-HT$_{2A}$, 5-HT$_{2B}$, and 5-HT$_{2C}$ receptors and the inverse agonistic and antagonistic activities of 5-HT$_{2A}$ receptors were detected; in the *in vivo* efficacy studies, the pharmacodynamic effects of PCC03039 on DOI-induced rat head-twitch model and MK-801-induced rat hyperlocomotion model were observed. The results of the studies showed that the affinities of PCC03039 for the 5-HT$_2$ receptors were comparable to those of the marketed drug pimavanserin, however, the inverse agonistic and antagonistic activities of PCC03039 for the 5-HT$_{2A}$ receptor were significantly improved, with IC$_{50}$ values of 2.11 nM and 1.33 nM, which were 20-fold and 21-fold higher than that of pimavanserin, respectively. PCC03039 could dose-dependently inhibit DOI-induced head-twitch and MK-801-induced hyperlocomotion in SD rats, and the pharmacodynamic effect was significantly better than pimavanserin at the equimolar dose. The above results show that PCC03039 has better pharmacodynamic activity *in vitro* and *in vivo* than pimavanserin, and has good druggability from the perspective of pharmacodynamics.

Keywords: Parkinson's disease psychosis, 5-HT$_{2A}$ receptor, inverse agonist, PCC03039

1 Introduction

Parkinson's disease (PD) is a common neurodegenerative disease and the average onset age is about 60 years [1]. At present, there are about 4 to 6 million PD patients in the world, and the incidence of PD and the number of patients are increasing year by year with the progress of population aging. About 50% of PD patients would develop severe symptoms of hallucinations or delusions, known as Parkinson's disease psychosis (PDP) [2,3]. PDP has the characteristics of high morbidity and long-lasting process, which has caused a heavy burden to the society and family. The pathophysiological mechanism of PDP remains unclear, and upregulation of the postsynaptic 5-HT$_{2A}$ receptor pathway in the cerebral cortex of PD patients is thought to play a central role in psychotic symptoms, especially visual hallucinations [4,5]. Antipsychotic drugs such as clozapine and quetiapine may have moderate short-term effects on PDP, but may lead to adverse reactions such as cognitive deterioration, tremor aggravation, and dyskinesia [6,7]. Therefore, there is an urgent need for PDP treatment drugs with less adverse reactions and clear therapeutic effects.

Pimavanserin (Nuplazid) was a 5-HT$_{2A}$ receptor inverse agonist developed by Acadia Pharmaceuticals, and was approved by the U.S. Food and Drug Administration (FDA) for the treatment of hallucinations and delusions associated with PDP in 2016 [8]. The results of clinical trials showed that the effect of pimavanserin was positively correlated with the dose and plasma concentration, but it could also prolong the QT interval in a dose-dependent manner, as shown in Figure 1 [9–11]. Therefore, the risk of cardiotoxicity of pimavanserin limited its use of higher doses in the clinic, and pimavanserin could not provide the best therapeutic effect for patients at its current clinical dose. In order to reduce the risk of cardiotoxicity of pimavanserin, Shandong Luye Pharmaceutical Co., Ltd. (Luye Pharma) optimized the structure of pimavanserin, designed and synthesized more than 200 compounds, and obtained the candidate compound PCC03039 after preliminary screening. The chemical structure of PCC03039 is shown in Figure 2. In this paper, the pharmacodynamics of PCC03039 was evaluated by *in vitro* and *in vivo* experiments to provide the evidence for its druggability and further clinical trial application.

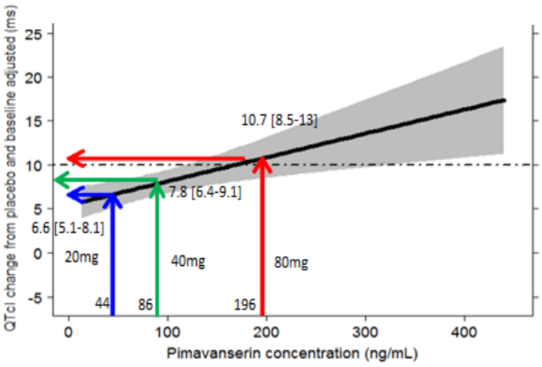

Figure 1 A single regression linear model between QTc and pimavanserin concentration [11]

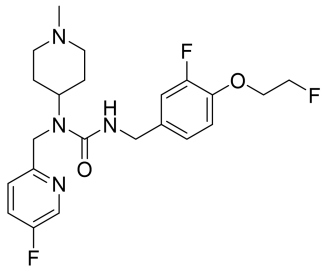

Figure 2 Chemical structure of PCC03039

2 Experimental materials

2.1 Cell line

HEK293 human embryonic kidney cells 5-HT_{2A}-HEK293, 5-HT_{2B}-HEK293, and 5-HT_{2C}-HEK293, which stably expressing human 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptors, respectively were supplied by WuXi AppTec (Shanghai) Co, Ltd. Mouse embryonic fibroblasts NIH3T3-5HTR2A-Luc2, stably expressing human 5-HT_{2A} receptor, was provided by KYinno Biotechnology (Beijing) Co., Ltd. The above cells were cultured in DMEM medium containing 10% fetal bovine serum (FBS) at 37°C and 5% CO_2.

2.2 Animals

Sprague-Dawley (SD) rats, male, 200-220 g, purchased from Jinan Pengyue Experimental Animal Technology Co., Ltd., animal production license number: SCXK (LU) 20190003. The rats were raised at a temperature of (21 ± 2)°C, humidity 30-70%, 12 hours of alternating light day and night in a specific pathogen-free (SPF) standard animal room and provided sufficient SPF animal-specific sterile feed and sterile deionized water. All animal-related experimental protocols and operations have been approved by the Animal Ethics Committee of Luye Pharma.

2.3 Chemicals and main reagents

PCC03039, white powder, purity 99.4%, provided by Luye Pharma, batch number SJC554-0093, molecular formula $C_{22}H_{27}F_3N_4O_2$, relative molecular mass 436.5, stored in the dark at 2 ~ 8°C. Drug solution preparation: an appropriate amount of the drug was weighed, dissolved and diluted to the desired concentrations with dimethyl sulfoxide (DMSO) or normal saline (0.9% NaCl injection), and drug solutions were prepared when used.

Pimavanserin, white powder, purity greater than 98.3%, provided by MedChemExpress, batch number HY-14557A, molecular formula $C_{25}H_{34}FN_3O_2$, relative molecular mass 427.6, stored at -20°C. Drug solution preparation: an appropriate amount of the drug was weighed, dissolved and diluted to the desired concentrations with DMSO or normal saline, and drug solutions were prepared when used.

2,5-Dimethoxy-4-iodophenylpropane hydrochloride (DOI), batch number 024M4613V, dizocycline hydrogen maleate (MK-801), batch number 105M4606V, both white powders, purchased from Sigma, stored at room temperature, dissolved and diluted to the desired concentrations with normal saline.

3 Experimental Method

3.1 Affinity of PCC03039 to 5-HT$_2$ receptor

In the 5-HT$_{2A}$ receptor affinity assay, the initial concentrations of PCC03039 and pimavanserin were 1000 nM, which were diluted to 0.0128 nM by a 5-fold gradient, with a total of 8 concentrations; the initial concentration of the positive compound Ketanserin was 50 nM, which was diluted by a 4-fold gradient to 0.003 nM, a total of 8 concentrations. In the 5-HT$_{2B}$ receptor affinity assay, the initial concentrations of PCC03039, pimavanserin and the positive compound (±) DOI were all 10000 nM, which were diluted to 0.61 nM by 4-fold gradient, with a total of 8 concentrations. In the 5-HT$_{2C}$ receptor affinity assay, the initial concentrations of PCC03039 and pimavanserin were 1000 nM, which were diluted to 0.0128 nM by a 5-fold gradient, with a total of 8 concentrations; the initial concentration of the positive compound SB-206553 was 1000 nM, which was diluted by a 4-fold gradient to 0.061 nM, a total of 8 concentrations.

5-HT$_{2A}$-HEK293, 5-HT$_{2B}$-HEK293, and 5-HT$_{2C}$-HEK293 cells were digested with trypsin, washed with PBS phosphate buffer, centrifuged at 3000 rpm for 5 min at room temperature, and the cells were collected and added with cell membrane extraction buffer, then cells were disrupted with a sonicator in an ice-water bath. The membrane protein solutions were obtained and the concentrations were determined. During the tests, add 100 μL of the prepared 5-HT$_{2A}$, 5-HT$_{2B}$, and 5-HT$_{2C}$ membrane receptor solutions to a 96-well (conical bottom) polypropylene reaction plate (Agilent), and the receptor contents were 10, 30, and 2.5 μg/100 μL/well respectively; transfer 1 μL of diluted PCC03039, pimavanserin or the corresponding positive compound to the reaction plate, and transfer 1 μL of the highest concentration of positive compound and 1 μL of DMSO to the reaction plate, respectively as low-signal non-specific binding wells (LC, Low control) and high signal total binding wells (HC, High control), respectively; add 100 μL of the corresponding prepared radioligand solutions ([3H]-Ketanserin, [3H]-LSD, [3H]-Mesulergine); After sealed with sealing film, the plates were placed on a shaker and incubated at 300 rpm for 1 h at room temperature; the GF/C filter plates (PerkinElmer) were soaked in 50 μL of 0.3% polyethyleneimine (PEI) for 0.5 h; after the incubation, the reaction solution in 96-well plates were collected into the GF/C filter plates, washed 4 times with washing buffer, 250 μL each time; the GF/C plates were placed in a 50°C oven to dry for 1 h, the bottom was sealed, and each well was added with 50 μL of Microscint-20 scintillation fluid, seal the plate with a transparent sealing film, and use MicroBeta2 (PerkinElmer) to read the plates. All groups had duplicate wells. The inhibition rates were calculated according to the following formula, inhibition rate % = (1-(sample well signal-LC signal)/(HC signal-LC signal)) × 100%, and "log(inhibitor) vs response-variable slope" in GraphPad Prism 5.0 was used to dose-inhibition ratio fitting, and the IC$_{50}$ and Ki values were calculated.

3.2 Inverse agonistic activity of PCC03039 on 5-HT$_{2A}$ receptor

The inverse agonism of PCC03039 on 5-HT$_{2A}$ receptor was detected using NIH3T3-5HTR2A-Luc2 cells and receptor selection and amplification techonology (R-SAT) [12]. The initial concentration of PCC03039 and pimavanserin was 10000 nM, which was diluted to 0.32 nM by a 3.16-fold gradient, with a total of 10 concentrations. NIH3T3-5HTR2A-Luc2 cells in logarithmic growth phase were collected and seeded at 1×10^3cells/180 μL/well in 96-well culture plates, and cultured at 37°C for 24 h. Twenty microliters of the above-diluted PCC03039 or pimavanserin were added to the corresponding wells, add 20 μL PBS as a high signal control well (HC), and add 10 μL PBS to a blank well as a low signal control well (LC). All groups had duplicate wells. The plates were incubated at 37°C for 5 days. On the 6^{th} day, the plates were taken out and placed at room temperature for 30 minutes, then 200 μL Bright-Glo™ Luciferase solution were added to each well, and incubated at room temperature for 20 minutes in the dark, shaked once every 5 minutes. The fluorescence luminescence intensity was detected on a multifunctional microplate reader. The fluorescence intensity inhibition rate was calculated according to the following formula: inhibition rate% = (1-(Lum_{sample} - Lum_{LC})/(Lum_{HC}-Lum_{LC}))×100%, and "log(inhibitor) vs response-variable slope" in GraphPad Prism 5.0 was used to dose-inhibition ratio fitting, and the IC$_{50}$ values were calculated.

3.3 Antagonistic activity of PCC03039 on 5-HT$_{2A}$ receptor

5-HT$_{2A}$-HEK293 cells were used to detect the antagonism of PCC03039 on 5-HT$_{2A}$ receptors. The antagonism of PCC03039 on 5-HT$_{2A}$ receptors could cause the changes in downstream calcium signaling pathways, which were detected by fluorescence imaging plate reading (FLIPR) in this study. 5-HT$_{2A}$-HEK293 cells in logarithmic growth phase were collected and diluted with

medium to 5×10^5 cells/mL, and seeded in 384-well culture plates at 2×10^4 cells/40 μL/well. After cultured at 37°C for 24 h, media was removed from the cell plates, and 20 μL assay buffer (20 mM HEPES, 1×HBSS, 0.5% BSA) and 20 μL 2× Fluo-4 detection reagent (8 μM Fluo-4, 12.5 mM probenecid) were added, then incubated at 37°C for 50 min and at room temperature for 10 min. In the 384-compound plate, the positive compound (methotrexate maleate), PCC03039, and pimavanserin were serially diluted, and each well contained 30 μL of detection solution after dilution. The initial concentration of methotrexate maleate was 10,000 nM, which was diluted to 0.508 nM by 3-fold concentration gradient, with a total of 10 concentrations. The initial concentration of PCC03039 and pimavanserin was 1000 nM, which were diluted to 0.051 nM by 3-fold concentration gradient, with a total of 10 concentrations. At the same time, the highest concentration of the positive compound was used as the low signal control well (LC), and 0.5% DMSO was used as the high signal control well (HC). The compound plate, cell plate and pipette tip were put into the FLIPR instrument, 10 μL of compound were transferred from the compound plate to the cell plate, and the fluorescence relative absorbance (RLU) was read. The RLU inhibition rate was calculated according to the following formula: inhibition rate% = (1-(RLU_{sample} - RLU_{LC})/(RLU_{HC} - RLU_{LC}))×100%, and "log(inhibitor) vs response-variable slope" in GraphPad Prism 5.0 was used to dose-inhibition ratio fitting, and the IC_{50} values were calculated.

3.4 Effects of PCC03039 on DOI-induced head-twitch in rats

The head-twitch model of rat induced by 2,5-dimethoxy-4-idophenyl-2-aminoprpane hydrochloride (DOI) was used to investigate the pharmacodynamic activity of PCC03039 *in vivo*. Sixty-four SD rats were randomly divided into 8 groups according to the body weight: normal saline (NS) group, model group, 6.0 μmol/kg pimavanserin group, and 0.375, 0.75, 1.5, 3.0, 6.0 μmol/kg PCC03039 groups, with 8 animals in each group. Rats were fasted from 17:00 the day before the test and acclimatized in the testing laboratory for at least 1 h on the day of the test. After the acclimatization, the rats were given the corresponding dose of pimavanserin or PCC03039 by a single gavage, and the control group and the model group were given the same volume of normal saline, and the administration volume was 1 mL/200 g. One hour after administration, the rats in the NS group were given normal saline, and the rats in the other groups were given 2.5 mg/kg DOI by intraperitoneal injection. Immediately after the injection of DOI, the head twitches of the rats were observed and recorded within 30 min (1.0-1.5 h after administration). Observations were randomly and double-blind, and the interference of human factors were excluded. The experimental data were expressed as mean ± standard error (Mean ± SEM), and SPSS statistics 20.0 software was used for one-way analysis of variance ANOVA to compare the differences in the number of head twitches among the groups. All statistical tests were two-sided, and $P < 0.05$ indicated statistical significance.

3.5 Effects of PCC03039 on MK-801-induced hyperlocomotion in rats

The hyperlocomotion model of rat induced by dizocycline hydrogen maleate (MK-801) was used to investigate the pharmacodynamic activity of PCC03039 *in vivo*. Forty-eight SD rats were randomly divided into 6 groups according to body weight: normal saline (NS) group, model group, 6.0 μmol/kg pimavanserin group, and 0.67, 2.0, 6.0 μmol/kg PCC03039 groups, with 8 animals in each group. Rats were fasted from 17:00 the day before the test. On the day of the test, the rats were given the corresponding dose of pimavanserin or PCC03039 by a single gavage, and the control group and the model group were given the same volume of normal saline, and the administration volume was 1 mL/200 g. At 45 min after administration, the rats in the normal group were given normal saline, and the rats in the other groups were given 0.3 mg/kg MK-801 by intraperitoneal injection. Five minutes after the injection of MKI-801, the rats were put into the detection box to acclimatization for 10 minutes, and then the TopScan monitoring system was used to record and analyze the locomotor activity distance (mm) of the rats in each group within 1.0-1.25 h after administration, and the Reduction Rate (RR) of locomotor activity distance was calculated according to the following formula: RR = (Activity distance of model group-Activity distance of treatment group)/Activity distance of model group*100%. During the test, the rats of the same group were avoided to be placed in the eight detection rooms in each round, and at least one NS group rat was placed in each round of test to prevent mutual interference. At the end of each ground of tests, the feces were cleaned to avoid the influence of irrelevant interfering factors (odor, etc.) on the rat activity. The test data were expressed as Mean ± SEM, and SPSS statistics 20.0 software one-way analysis of variance ANOVA was used to compare the differences between groups at each time point. All tests were two-sided

tests, $P < 0.05$ indicated statistical significance.

4 Experimental Results

4.1 Affinity of PCC03039 to 5-HT$_2$ receptor

The affinity test results of PCC03039 for different 5-HT receptors are shown in Figure 3. The IC_{50} values of affinity of PCC03039 for 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptor were 0.42, 299, and 3.79 nM, respectively, and the Ki values were 0.20, 158.7, and 1.87 nM, respectively. The IC_{50} values of affinity of pimavanserin for 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptor were 0.37, 352, 3.61 nM, respectively, and the Ki values were 0.15, 166.3, 2.16 nM, respectively. The above results indicated that the selectivity and affinity of PCC03039 for different 5-HT$_2$ receptors were comparable to pimavanserin.

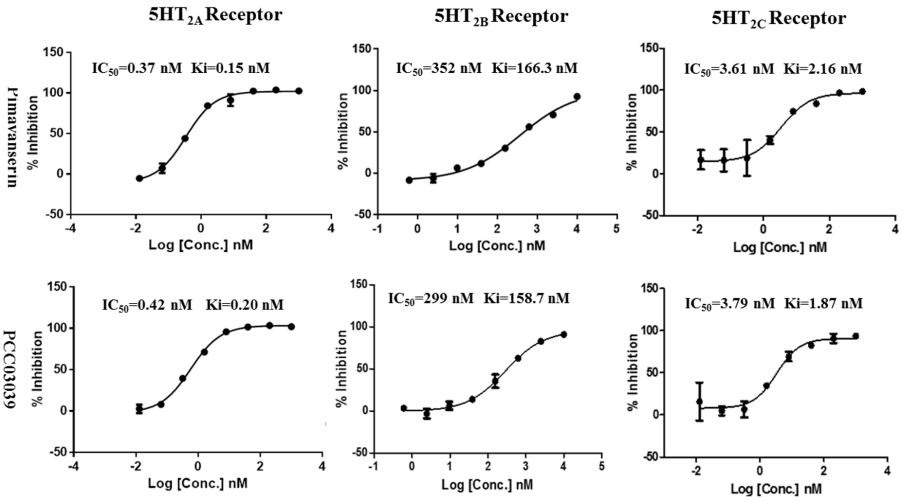

Figure 3 Affinity of PCC03039 to different 5-HT receptors

4.2 Inverse agonistic activity of PCC03039 on 5-HT$_{2A}$ receptor

The test results of the inverse agonistic activity of PCC03039 on 5-HT_{2A} receptor are shown in Figure 4. The IC_{50} value of inverse agonistic activity of PCC03039 on 5-HT_{2A} receptor was 2.11 nM, and the IC_{50} value of inverse agonistic activity of pimavanserin was 42.26 nM. The above results indicated that the inverse agonistic activity of PCC03039 on the 5-HT_{2A} receptor was significantly enhanced *in vitro*, which was about 20 times that of pimavanserin.

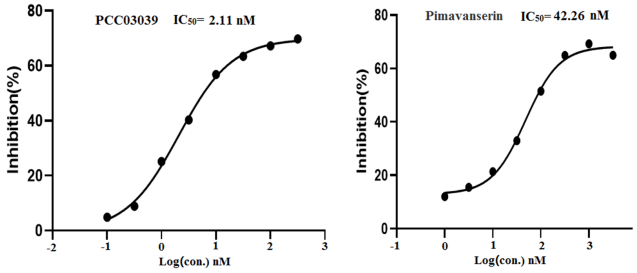

Figure 4 Inverse agonism of PCC03039 on 5-HT_{2A} receptors

4.3 Antagonistic activity of PCC03039 on 5-HT$_{2A}$ receptor

The results of the antagonistic activity of PCC03039 on 5-HT_{2A} receptor are shown in Figure 5. The IC_{50} value of PCC03039 for 5-HT_{2A} receptor antagonism was 1.33 nM, and the IC_{50} value of pimavanserin was 28.45 nM. The antagonistic activity of 5-HT_{2A} receptor was significantly enhanced, which was about 21 times that of pimavanserin.

4.4 Effects of PCC03039 on DOI-induced head-twitch in rats

One hour after a single intragastric administration, the number of head twitches of the rats within 0.5 h were observed and recorded immediately after intraperitoneal injection of DOI.

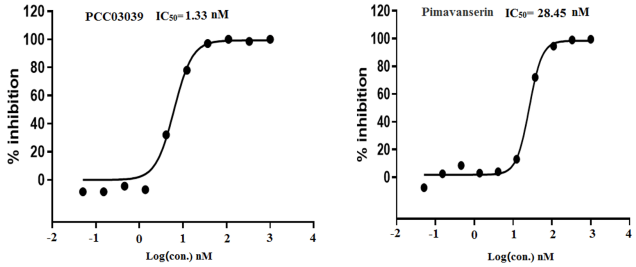

Figure 5 Antagonism of PCC03039 on 5-HT_{2A} receptor

The results are shown in Figure 6. Compared with the NS control group, the number of head twitches in the model group was significantly increased ($P<0.05$), with the mean of 2.9 and 24.3 times, respectively, indicating that the model was successfully established. Compared with the model group, the number of head twitches in the 6 μmol/kg pimavanserin group was significantly decreased ($P<0.05$), with a mean of 6 times. Compared with the model group, there was no significant difference in the number of head twitches in the 0.375 μmol/kg PCC03039 group, with a mean of 22 times. The head twitches in the 0.75-6.0 μmol/kg PCC03039 groups were 0.5, 2, 3.3, and 10.7 times, respectively, which were in a dose-dependent manner and significantly lower than those in the model group ($P<0.05$). The numbers of head twitches in the PCC03039 group at 0.75, 1.5, and 3 μmol/kg were similar to that in the 6 μmol/kg pimavanserin group, and there was no significant difference ($P>0.05$), but numerically, the number of head twitches in the 1.5 μmol/kg PCC03039 group was closer to that in the 6 μmol/kg pimavanserin group. The above results showed that PCC03039 could dose-dependently inhibit the number of head twitches induced by DOI in rats, and the pharmacodynamic effect of PCC03039 was significantly higher than that of pimavanserin at the same molar dose.

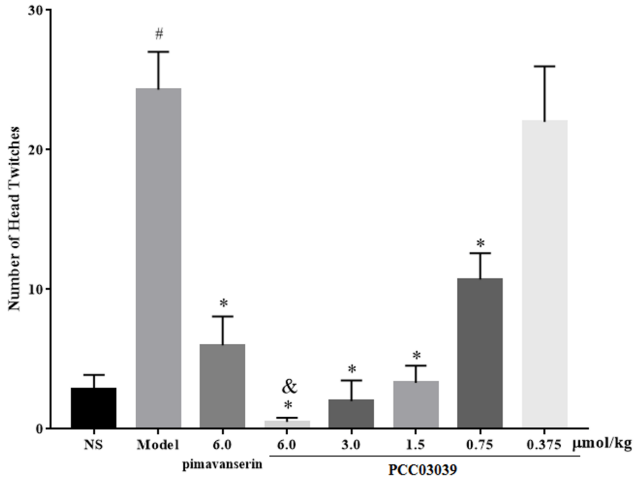

Figure 6 Effects of PCC03039 on DOI-induced head twitch in rats

SD rats were given NS, 6.0 μmol/kg pimavanserin, and 0.375, 0.75, 1.5, 3.0, 6.0 μmol/kg PCC03039 by a single gavage, and the number of head twitches induced by DOI was observed 1.0-1.5 h after administration, n= 8. $^{\#}P<0.05$, compared with the NS group; $^{*}P<0.05$, compared with the model group; & $P<0.05$, compared with the PCC03039 group.

4.5 Effects of PCC03039 on MK-801-induced hyperlocomotion in rats

Forty-five minutes after a single intragastric administration, the model was established by intraperitoneal injection of MK-801, and the locomotor activity distances of the rats in each group within 1.0-1.25 h after administration were observed. The results are shown in Table 1 and Figure 7. The mean locomotor activity distances of rats in NS group and model group were 547 mm and 8827 mm, respectively. Compared with NS group, the mean locomotor activity distances of rats in model group was significantly increased ($P<0.05$), indicating that the model was successfully established. The locomotor activity distances of rats in the 6.0 μmol/kg pimavanserin group were reduced, with an average value of 4878 mm, and the reduction rate was 44.7%, but there was no statistical difference compared with the model group (P>0.05). At

doses of 0.67, 2.0, and 6.0 μmol/kg, PCC03039 could reduce the locomotor activity distances of rats, and the reduction rates were 6.3%, 42.5%, and 93.0%, respectively, and there was a statistical difference at the dose of 3.0 mg/kg ($P<0.05$). The above results showed that PCC03039 was more effective than pimavanserin in MK-801-induced hyperlocomotion model in rats.

Table 1 Effects of PCC03039 on MK-801-induced hyperlocomotion in rats

Groups	Locomotor activity distances (mm)	Reduction rate (RR) of locomotor activity distance
NS	547.0 ± 253.1	-
Model	8827.0 ± 2541.0$^{\#}$	-
Pimavanserin 6.0 μmol/kg	4878.0 ± 1654.0	44.7%
PCC03039 0.67 μmol/kg	8273.0 ± 2190.0	6.3%
PCC03039 3.0 μmol/kg	5077.0 ± 1993.0	42.5%
PCC03039 6.0 μmol/kg	618.0 ± 252.1*	93.0%

Note: $^{\#}P<0.05$, compared with the NS group; $^{*}P<0.05$, compared with the Model group.

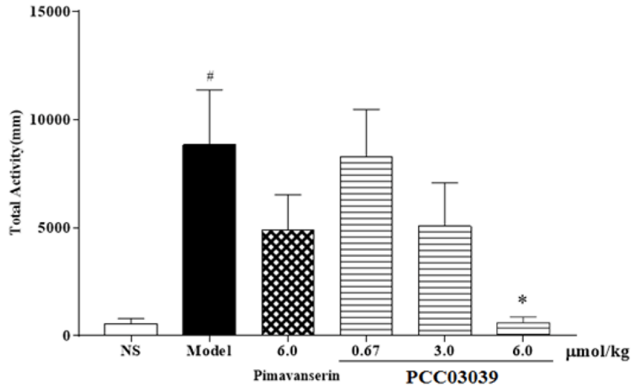

Figure 7 Effects of PCC03039 on MK-801-induced hyperlocomotion in rats

SD rats were given NS, 2.8 mg/kg pimavanserin, or 0.67, 3.0, 6.0 μmol/kg PCC03039 by a single gavage. After induction with MK-801, the locomotor activity distances of rats were detected from 1.0 to 1.25 h after administration, n = 8. $^{\#}P<0.05$, compared with the NS group; $^{*}P<0.05$, compared with the model group.

5 Discussion and conclusion

PDP is a non-motor symptom that occurs in the progress of Parkinson's disease, including a series of positive clinical manifestations such as hallucinations, delusions, false hallucinations or delusions [13, 14]. Pimavanserin is a highly selective 5-HT_{2A} receptor inverse agonist that exerts pharmacological effects by inhibiting the intrinsic activity of serotonin receptors [15, 16]. Pimavanserin has no activity on dopamine receptors and does not aggravate the motor symptoms of Parkinson's disease symptoms, and has become the first-line drug for the treatment of PDP [17, 18]. The results of clinical trials of pimavanserin showed that its pharmacodynamic effect was positively correlated with dose and blood concentration, but at the same time, it could prolong the QT interval in a dose-dependent manner [19, 20]. At the maximum clinical dose of 34 mg/day, the QT interval was prolonged by about 8 ms, and when the dose was doubled, the QT interval was prolonged by about 13.5 ms. Therefore, the existence of cardiotoxicity limits the clinical dose of pimavanserin, which could not allow patients to obtain the maximum clinical therapeutic effect.

To reduce the potential cardiac safety risk of pimavanserin, the candidate compound PCC03039 was obtained by structural optimization based on pimavanserin. The results of *in vitro* affinity studies showed that the affinity and selectivity of PCC03039 for 5-HT_2 receptors were comparable to those of pimavanserin, however, the inverse agonistic and antagonistic activities of PCC03039 for 5-HT_{2A} receptors were significantly enhanced, which were more than 20 times that of pimavanserin. The results of *in vivo* pharmacodynamic studies showed that PCC03039 exhibited pharmacodynamic activities related to the pharmacological mechanism of action in both the DOI-induced rat head-twitch model and the MK-801-induced rat hyperlocomotion model, and was significantly better than pimavanserin. In the sensitive DOI-induced rat head-twitch model, the pharmacodynamic effect of PCC03039 was approximately 4 times that of pimavanserin.

The results of previous *in vitro* studies showed that the inhibitory effect of PCC03039 on hERG current was slightly higher than that of pimavanserin, with IC_{50} values of 1.82 μM and 1.09 μM, respectively, and the drug concentrations in the heart were similar. However, the results of this study showed that the *in vitro* and *in vivo* efficacy of PCC03039 was significantly higher than that of pimavanserin. Therefore, it is expected that the clinical dose of PCC03039 is 1/4-1/2 of the clinical dose of pimavanserin, which will be significantly lower. The reduction of the clinical dose of PCC03039 might reduce its cardiac safety risk, or the clinical dose could be increased under the same cardiac safety risk to achieve the purpose of improving the clinical therapeutic effect.

In conclusion, the *in vitro* and *in vivo* pharmacodynamics of a new 5-HT_{2A} receptor inverse agonist PCC03039 was evaluated, which showed that PCC03039 had significantly better *in vitro* and *in vivo* pharmacodynamics than pimavanserin, and its clinical dose was expected to be significantly reduced. which could reduce the risk of cardiac safety. From the perspective of pharmacodynamics, PCC03039 has good druggability, which supports its entry into clinical trials to further study its safety and efficacy.

Author contributions

Sujie Liu, Xin Wang, Huan Gao, conducted specific experimental operations and data analysis of experimental results. Guangying Du, Jingwei Tian and Liang Ye conceived and designed the current study. Sujie Liu wrote the manuscript. Sujie Liu and Guangying Du confirm the authenticity of all raw data.

Acknowledgements

Thank you to all colleagues who have worked hard and contributed.

Declaration of competing interest

Authors declare no conflict of interest.

Ethical statement

All animal-related experimental protocols and operations have been approved by the Animal Ethics Committee of Luye Pharma.

References

[1] Kalia LV and Lang DAE. Parkinson's disease. Lancet, 2015, **386**(9996): 896-912. https://doi.org/10.1016/S0140-6736(14)61393-3

[2] Fénelon G and Alves G. Epidemiology of psychosis in Parkinson's disease. Journal of the Neurological Sciences, 2010, **289**(1-2): 12-17. https://doi.org/10.1016/j.jns.2009.08.014

[3] Bloem BR, Okun MS and Christine K. Parkinson's disease. Lancet, 2021, **397**(10291): 2284-2303. https://doi.org/10.1016/S0140-6736(21)00218-X

[4] Meltzer HY, Mills R, Revell S, *et al*. Pimavanserin, a Serotonin2A Receptor Inverse Agonist, for the Treatment of Parkinson's Disease Psychosis. Neuropsychopharmacology, 2010, **35**(4): 881-892. https://doi.org/10.1038/npp.2009.176

[5] Michaud V. Cannabis Dopaminergic Effects Induce Hallucinations in a Patient with Parkinson's Disease. Medicina, 2021, **57**(10): 1107. https://doi.org/10.3390/medicina57101107

[6] Breier A, Buchanan RW, Kirkpatrick B, *et al*. Effects of clozapine on positive and negative symptoms in outpatients with schizophrenia. American Journal of Psychiatry, 1994, **15**(1): 20-26. https://doi.org/10.1176/ajp.151.1.20

[7] Cheer SM and Wagstaff AJ. Quetiapine. Cns Drugs, 2004, **18**(3): 173-199. https://doi.org/10.2165/00023210-200418030-00004

[8] Mcfarland K, Price DL and Bonhaus DW. Pimavanserin, a 5-HT2A inverse agonist, reverses psychosis-like behaviors in a rodent model of Parkinson's disease. Behavioural Pharmacology, 2011, **22**(7): 681-692. https://doi.org/10.1097/FBP.0b013e32834aff98

[9] Cummings J, Isaacson S, Mills R, *et al*. Pimavanserin for patients with Parkinson's disease psychosis: a randomised, placebo-controlled phase 3 trial. Lancet, 2014, **383**(9916): 533-540. https://doi.org/10.1016/S0140-6736(13)62106-6

[10] Hacksell U, Burstein ES, Mcfarland K, *et al*. On the Discovery and Development of Pimavanserin: A Novel Drug Candidate for Parkinson's Psychosis. Neurochemical Research, 2014, **39**(10): 2008-2017. https://doi.org/10.1007/s11064-014-1293-3

[11] Kumi KA, Zhu H and Mehta MU. Center for Drug Evaluation and Research, 2016. https://www.accessdata.fda.gov/drugsatfda_docs/nda/2016/207318Orig1s000ClinPharmR.pdf

[12] Vanover KE . Pharmacological and behavioral profile of N-(4-fluorophenylmethyl)-N-(1-methylpiperidin-4-yl)-N'-(4-(2-methylpropyloxy)phenylmethyl) carbamide (2R,3R)-dihydroxybutanedioate (2:1) (ACP-103), a novel 5-hydroxytryptamine(2A) receptor inverse agonist. Journal of Pharmacology & Experimental Therapeutics, 2006, **317**(2): 910-919. https://doi.org/10.1124/jpet.105.097006

[13] Dashtipour K, Gupta F, Hauser RA, *et al*. Pimavanserin Treatment for Parkinson's Disease Psychosis in Clinical Practice. Parkinson's Disease, 2021, **2021**(10): 1-10. https://doi.org/10.1155/2021/2603641

[14] Sammartino F, Marsh R, Rezai A, *et al*. Non-motor effects of subthalamic nucleus stimulation in Parkinson patients. Brain Imaging and Behavior, 2022, **16**(1): 161-168. https://doi.org/10.1007/s11682-021-00487-8

[15] Burstein ES. Relevance of 5-HT2A Receptor Modulation of Pyramidal Cell Excitability for Dementia-Related Psychosis: Implications for Pharmacotherapy. CNS Drugs, 2021, **2021**(1): 1-15. https://doi.org/10.1007/s40263-021-00836-7

[16] Dragana BK, Istvan B, Liu IY, *et al*. ENHANCE: Phase 3, Randomized, Double-Blind, Placebo-Controlled Study of Adjunctive Pimavanserin for Schizophrenia in Patients With an Inadequate Response to Antipsychotic Treatment. Schizophrenia Bulletin Open, 2022, **2022**(1): 1-6. https://doi.org/10.1093/schizbullopen/sgac006

[17] Kwan C, Frouni I, Nuara SG, *et al*. Combined 5-HT2A and mGlu2 modulation for the treatment of dyskinesia and psychosis in Parkinson's disease. Neuropharmacology, 2021, **186**(10): 65-84. https://doi.org/10.1016/j.neuropharm.2021.108465

[18] Dashtipour K, Gupta F, Hauser RA, *et al*. Pimavanserin Treatment for Parkinson's Disease Psychosis in Clinical Practice. Parkinson's Disease, 2021, **2021**(10): 1-10. https://doi.org/10.1155/2021/2603641

[19] Tampi RR, Tampi DJ, Young JJ, *et al*. Evidence for using pimavanserin for the treatment of Parkinson's disease psychosis. World Journal of Psychiatry, 2019, **9**(3): 47-54. https://doi.org/10.5498/wjp.v9.i3.47

[20] Steinberg M. Update on Pharmacological Treatment of Neuropsychiatric Symptoms of Dementia. Current Geriatrics Reports, 2021, **10**(2): 51-57. https://doi.org/10.1007/s13670-021-00356-8