

# Discovery and Characterization of TAS-4: A Potent and Selective Metabotropic glutamate receptor 4 positive allosteric modulator improves movement in rodent models of Parkinson's disease.

Dube Aakanksha, Chaudhary Sumit, Singh Nripendra and Upasani Devidas Chandrashekhar

**Abstract**— Parkinson's disease (PD) is a very serious neurological disorder, and current methods of treatment fail to achieve long-term control. Previous studies suggest that stimulation of the metabotropic glutamate receptor 4 (mGluR4) represents a promising new approach to the symptomatic treatment of the Parkinson's disease (PD). Preclinical models using both agonists and positive allosteric modulators of mGluR4 have demonstrated the potential for this receptor for the treatment of PD.

In the present study, we describe the pharmacological characterization of an mGluR4 PAM, N-(2, 4-dichlorophenyl) pyridine-2-carboxamide (TAS-4), in several rodent PD models. TAS-4 are potent and selective mGluR4 PAM of the human mGluR4 receptor (EC<sub>50</sub>- 287.8 nM). TAS-4 showed efficacy alone or when administered in combination with L-DOPA. When administered alone, TAS-4 exhibited efficacy in reversing haloperidol-induced catalepsy. In addition, Acute TAS-4 dose-dependently potentiated contralateral turning behavior induced by a threshold dose of L-3, 4-dihydroxyphenylalanine (L-DOPA, 4 mg/kg i.p.), a classical test for antiparkinson drug screening. Sub chronic (28 days, twice a day) TAS-4 (10 mg/kg i.p.) + L-DOPA (4 mg/kg i.p.) did not induce sensitization to turning behavior or abnormal involuntary movements during the course of treatment. Moreover, while sub chronic administration of a fully effective dose of L-DOPA (8 mg/kg i.p.) significantly induce sensitization to turning behavior or abnormal involuntary movements.

Results showed that TAS-4, in association with a low dose of L-DOPA, displayed antiparkinsonian activity similar to that produced by a full dose of L-DOPA without exacerbating abnormal motor side effects.

**Key words:** TAS-4, Metabotropic Glutamate Receptor 4-Positive Allosteric Modulator (mGluR4 PAM), 6-OHDA (6-hydroxydopamine), L-DOPA (levodopa), Parkinson's disease (PD), Abnormal Involuntary Movements

## 1 Introduction

Parkinson's disease (PD) is characterized by degeneration of nigrostriatal dopaminergic neurons, which results in an imbalance in the direct and indirect pathways. Pharmacological treatment of PD by improving dopaminergic transmission through the enhancement of dopamine release with L-DOPA or direct activation of dopamine D<sub>2</sub>/D<sub>3</sub> receptors has been relatively successful but often has been accompanied by serious side effects, including dyskinesias, somnolence, and compulsive behaviour [1-3].

A major goal of Parkinson's disease (PD) drug discovery is development of nondopaminergic therapies that improve motor deficits without the liability for the adverse chronic effects of standard dopaminergic drugs. An alternative non-

*Dr. Chandrashekhar Devidas Upasani Department of Pharmacology, Faculty of pharmacy, Shri Neminath Jain Bramhacharyashram's Shriman Sureshdada Jain college of pharmacy Jain gurukul, Chandwad, Nashik,423101 Maharashtra,India*

*Sumit Chaudhary. Department of Pharmacology, Faculty of pharmacy, Shri Neminath Jain Bramhacharyashram's Shriman Sureshdada Jain college of pharmacy Jain gurukul, Chandwad, Nashik,423101 Maharashtra,India  
Sumit.pharmacology@gmail.com*

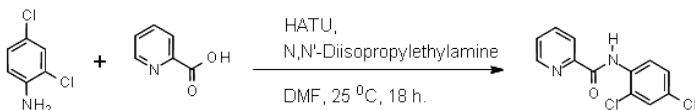
dopaminergic palliative treatment for PD is via activation of the metabotropic glutamate receptor 4 [4].

mGluR4 is a member of the Group C class of GPCRs and is one of eight known mGluRs (1 – 8). The mGluRs are subdivided into three groups according to their sequence homology, functional coupling and pharmacology (Group 1: mGluR 1 and 5; Group II: mGluR 2 and 3; Group III: mGluR 4, 6 – 8) [5]. During the progression of the disease, degeneration of DA neurons in the SNc leads to a reduction of nigrostriatal neurotransmission causing an imbalance between the direct/indirect pathways, via D1 and D2 receptors respectively. mGluR4 is expressed at the striatopallidal synapses and STN-SNr synapses. Its localization suggests it functions as a presynaptic heteroreceptor on GABAergic neurons, suggesting in turn that selective activation or positive modulation of mGluR4 would decrease GABA release in this synapse, thereby decreasing output of the indirect pathway and reducing or eliminating the PD symptoms [6].

Direct stimulation of the glutamate (orthosteric) binding site or indirect modulation of the glutamate response by a ligand

that binds at an allosteric site have been postulated as two related approaches to correct the abnormal basal ganglia circuitry in late-stage PD by decreasing GABA release and consequently attenuating glutamatergic overactivity [7]. Both orthosteric mGluR4 agonists such as L-AP-4 or LSP1-2111 [8] and mGluR4-positive allosteric modulators such as (-)-PHCCC [9] have been shown to improve motor function in preclinical rodent models of PD [10] Hence, mGluR4 is regarded as an attractive target for the identification of an adjunctive therapy to dopamine agonists and/or L-DOPA. Due to the highly conserved nature of the glutamate binding site, the search for potent and selective agonists of the mGluRs is considered to be highly challenging. Allosteric modulation of mGluRs [11] is widely accepted to be an attractive alternative to targeting the orthosteric binding site as it may offer several advantages. The allosteric site (or sites) is likely to be less well conserved across the different mGluR subtypes and hence the identification of selective ligands is expected to be more easily achievable. Early mGlu4 PAMs provided important proof-of-concept data suggesting antiparkinsonian-like efficacy in preclinical PD models, but had limited utility as in vivo tools because of unsuitable physicochemical properties (e.g., limited solubility, selectivity, central penetration and short half-life) for systemic dosing studies [9,12,13]

Using an alternative strategy, our group have identified highly selective and potent mGluR4 ligands that potentiate receptor activity via action at sites that are less highly conserved and topographically distinct relative to the glutamate binding site, termed positive allosteric modulators (PAMs). In an effort to search for novel mGluR4 PAMs, we screened a library of various structurally diverse synthetic compounds. Among active compounds identified, a compound with indene structure was chosen based on the novelty and ease of derivatives synthesis, and chemical modification of this molecule lead to the TAS-4 as a lead compound for novel mGluR4 PAMs (Fig. 1). TAS-4 chemically known as N-(2, 4-dichlorophenyl) pyridine-2-carboxamide. Here, we describe the pharmacologic characterization of TAS-4 (mGluR4 PAM). Using multiple rodent PD models, we show that TAS-4 exhibits efficacy when administered alone as well as in combination with L-DOPA.



**Fig 1** Synthesis scheme and chemical structure of TAS-4 (N-(2, 4-dichlorophenyl) pyridine-2-carboxamide).

## 2. MATERIALS AND METHODS

### 2.1. Animals

Male Wistar rats weighing 200 to 250 g were used for catalepsy

study. Rats were housed three or four per cage in a temperature and humidity-controlled environment, with food and water available ad libitum. They were maintained on a 12-h light/dark cycle (lights on 7:00 AM, lights off 7:00 PM), and all studies were conducted between 9:00 AM and 5:00 PM. Male Wistar rats weighing 290 to 310 g were used for unilateral 6-OHDA lesion experiments. Animals were housed under a normal 12-h light/dark cycle (lights on 7:00 AM, lights off 7:00 PM) with food and water available ad libitum. All behavioural testing was performed between 8:00 AM and 2:00 PM. These procedures involving the use of animals and their care were conducted in conformity with CPCSEA Guidelines. All studies involving animals were approved by Institutional animal ethical committee of SNJB College of pharmacy, Chandwad.

### 2.2. Drugs and Injections

Apomorphine hydrochloride, benserazide hydrochloride, haloperidol, L-DOPA methyl ester, and 6-hydroxydopamine hydrobromide were obtained from Sigma-Aldrich (St. Louis, MO). L-Glutamate was obtained from Tocris Bioscience (Ellisville, MO). The compounds were dissolved in dimethyl sulfoxide (DMSO) and added to medium to a final DMSO concentration of 0.1% for in vitro studies and were suspended in an aqueous solution of 1% Tween 80 and 0.5 % Carboxy methyl cellulose. 6-OHDA (Sigma-Aldrich, St. Louis, MO) was dissolved in saline containing 0.05% ascorbic acid and infused at a final concentration of 2 µg/µl (8 µg total). Benserazide and L-DOPA methyl ester were dissolved in 0.9% saline, whereas haloperidol were dissolved in 0.85% lactic acid (Sigma) and 0.5% Cremophor EL (Sigma-Aldrich, St. Louis, MO) respectively. Except for apomorphine and L-DOPA, which are unstable at neutral pH, all drug formulations were adjusted to a pH of approximately 7 by using 1 N sodium hydroxide. Drugs were administered in a volume of 1 ml/kg (i.p./s.c./i.v.) or 3 ml/kg (p.o.).

### 2.3. Synthesis of TAS-4

The chemical synthesis for the TAS-4 was completed by an in-house synthesis using the following method. N-(2,4-dichlorophenyl)pyridine-2-carboxamide-To a stirred solution of 2,4-dichloroaniline (1gm, 6.172 mmol) and pyridine-2-carboxylic acid (0.910 gm, 7.40 mmol) in dimethylformamide (5 ml) was added N,N-diisopropylethylamine (2.0 ml, 12.318 mmol) under nitrogen gas ((Fig. 1). HATU (O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate- 3.52 g, 9.258 mmol) was added in single portion and reaction mixture was stirred 16 hrs at room temperature. Reaction was quenched with ice-cold water (15 ml), solid was precipitated. Filtered through Buckner funnel, residue was washed with water followed by sat. Sodium bicarbonate solution to get rid of unreacted starting acid. Again washed with water, dried over high vacuum. The desired product was obtained as an off-white solid (1.3g, 81% yield): Rf 0.47 (20% EtOAc/hexanes); LCMS: single peak (M+1, 267.0), <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): 10.74 (br s, 1H), 8.77 (d, J

= 2.2 Hz, 1H), 8.67 (d, J = 8.0 Hz, 1H), 7.94 (dt, J = 1.8, 9.3 Hz, 1H), 7.52 (dt, J = 0.9, 7.5 Hz, 1H), 7.35 (d, J = 8.0, 8.8 Hz, 1H), 7.05 (dd, J = 8.4, 5.8 Hz, 1H).

## 2.4. Cell Culture

Human mGluR4/Gqi5/Chinese hamster ovary stably transfected cell lines using pIRES/Neo (Invitrogen) were grown in 90% Dulbecco's modified Eagle's medium (DMEM), 10% dialyzed fetal bovine serum (FBS), 100 units/ml penicillin/streptomycin, 20 mM HEPES, pH 7.3, 1 mM sodium pyruvate, 20 µg/ml proline, 2 mM glutamine, 400 µg/ml G418 sulfate (calbiochem), Rat mGluR4/HEK/G protein-regulated inwardly rectifying K (potassium) channel (GIRK) cells, as well as HEK/GIRK lines expressing rat mGluR2, mGluR3, mGluR7, mGluR8, and human mGluR6, were cultured in 45% DMEM, 45% Hams F-12, 10% FBS, 20 mM HEPES, 2 mM L-glutamine, 100 units/ml penicillin/streptomycin, 1X nonessential amino acids, 1 mM sodium pyruvate, 700 µg/ml G418, and 0.6 µg/ml puromycin. Rat mGluR1 and mGluR5 HEK cell lines were grown in DMEM, 10% FBS, 20mMHEPES, 2 mM L-glutamine, 1Xnonessential amino acids, 1 mM sodium pyruvate, and 500µg/ml G418.

### 2.4.1. Effect of TAS-4 in Calcium mobilization assays

Human mGluR4/Gqi5/Chinese hamster ovary cells (50,000 cells/100µl/well) were plated in black-walled, clear-bottomed, tissue culture-treated, 96well plates (Greiner Bio- One, Monroe, NC) in DMEM containing 10% FBS, 100 units/ml penicillin/streptomycin, and 1 mM sodium pyruvate (plating medium). The cells were grown overnight at 37°C in the presence of 5% CO<sub>2</sub>. The next day, the medium was removed and replaced with 20 µl of 1 µM Fluo-4 AM (Life Technologies F-14201) prepared as a 1 mM stock in DMSO and mixed in a 1:1 ratio with 10% (w/v) Pluronic acid F-127 and diluted in assay buffer (Hanks' balanced salt solution, 1%BSA, and 2.5 mM probenecid; Sigma) for 45 min at 37°C. Dye was removed and replaced with 100 µl of assay buffer. Potency of TAS-4 was determined by performing concentration-response curves (CRCs, 9 points, ranging from approximately 10 µM-1 nM at 0.5% final DMSO concentration) at human mGlu4 using this calcium assay in which the cells express the chimeric G protein Gqi5 to couple mGlu4 to calcium mobilization. Ca<sup>2+</sup> flux was measured by using Flexstation III (Molecular devices) of a fluorescence baseline for 15 s (excitation, 488 nm; emission, 535 ± 210 nm), 5X of stock of compounds was made so 20 µl of compounds were added to the cells. Glutamate was added by machine after 15 secs.

Calcium fluorescence was recorded as fold over basal fluorescence, and raw data were normalized to the maximal response to glutamate. Potency (EC<sub>50</sub>) and maximum response (percentage of Glu maximum) for compounds were determined by using a four-parameter logistical equation in Prism (GraphPad Software, Inc., San Diego, CA).

### 2.4.2. Effect of TAS-4 in GIRK-Mediated thallium flux assay

Cells were plated into 96-well, black-walled, clear-bottom, poly-D-lysine-coated plates (Greiner Bio-One) at a density of 50,000 cells/100 µl/well in DMEM containing 10% dialyzed FBS, 20 mM HEPES, and 100 units/ml penicillin/streptomycin (assay media). Plated cells were incubated overnight at 37°C in the presence of 5% CO<sub>2</sub>. The next day, the medium was removed from the cells, and 20 µl/well of 230 nM Fluo Zn2 (Invitrogen; prepared as a stock in DMSO and mixed in a 1:1 ratio with Pluronic acid F-127) in assay buffer [Hanks' balanced salt (Invitrogen) containing 20 mM HEPES, pH 7.3] was added to the plated cells. Cells were incubated for 1 hr at room temperature, and the dye was replaced with the 100 µl of assay buffer. Agonists were diluted in the following thallium buffer [gluconate buffer: 125 mM sodium gluconate, 1 mM magnesium sulfate, 1.8 mM calcium gluconate, 5 mM glucose, 12 mM thallium sulfate, and 10 mM HEPES, pH 7.3.] Appropriate baseline readings were taken (excitation, 470 ± 20 nm; emission, 540 ± 30 nm) using FlexstationIII (Molecular devices) and when included in an experiment, antagonists/potentiators were added in a 20-µl volume and incubated for 5 min before the addition of 10 µl of thallium buffer with or without agonist. After the addition of agonist, data were collected for an additional 5 min.

### 2.4.3. Selectivity of TAS-4 over rat mGluR1 and mGluR5

HEK cells expressing rat mGluR1 and mGluR5. The effects of TAS-4 on rat mGluR1 and mGluR5 were assessed by using calcium mobilization and measuring the glutamate concentration-response relationship in the presence and absence of 10 µM TAS-4. Using a double-addition protocol, TAS-4 was added to the cells, followed 5 min later by a full concentration response of glutamate. Shifts of the concentration-response relationship were used to assess potential potentiator (left shift of more than 2-fold) or antagonist (right shift of more than 2-fold or depression of the maximum response by at least 75%) activity of TAS-4. Compounds were further assessed for mGluR5 antagonist activity by performing a full concentration-response curve, starting at 10 µM and serially diluted it by using 1:3 dilutions, in the presence of an EC<sub>80</sub> concentration of glutamate.

### 2.4.4. Selectivity of TAS-4 over Rat mGluR2, mGluR3, mGluR7, and mGluR8 and Human mGluR6.

Compound activity at the rat group II and III mGluRs was assessed by using thallium flux through GIRKs as described above. Cells were plated at a density of 15,000 cells/20 l/well in assay media. The effects of TAS-4 were assessed by measuring the glutamate concentration-response relationship in the presence and absence of 10 µM of TAS-4. Using a double-addition protocol, TAS-4 was added to the cells, followed 5 min later by a full concentration-response of glutamate or, in the case of mGluR7, (2S)-2-amino-4-phosphonobutanoic acid. As above, shifts of the concentration-response relationship were used to assess potential potentiator (left shift of more

than 2-fold) or antagonist (right shift of more as 2-fold or depression of the maximum response by at least 75%) activity of TAS-4. Compounds were further assessed for human mGluR6 PAM activity by performing a full concentration-response curve, starting at 10  $\mu$ M and serially diluted it using 1:3 dilutions, in the presence of an EC20 concentration of glutamate.

## 2.5. In vivo pharmacokinetic profile of TAS-4

Male Wistar rats, weighing approximately 200 to 250 g, were used for pharmacokinetic studies. The animals were acclimated to their surroundings for approximately 1 week before dosing and provided food and water ad libitum. Parenteral administration of compounds to rats was achieved via a penile vein injection at a dose of 1 mg/kg (5% DMSO + 95% saline, 1 ml/kg, i.v.) and a dose volume of 1 ml/kg. Blood collections via the retro orbital puncture under isoflurane anesthesia were performed at predose, and 5, 15 and 30 min and 1, 2, 4, and 8 h post dose. Samples were collected into chilled EDTA-fortified tubes and centrifuged for 10 min at 5000 rpm (4°C), and the resulting plasma was aliquoted into 96-well plates for HPLC analysis. Pharmacokinetic parameters were obtained from noncompartmental analysis (WinNonLin, V5.3; Pharsight, Mountain View, CA) of individual concentration time profiles after the parenteral administration of a test article. For systemic exposure studies, measuring both systemic plasma and central nervous system tissue exposure, TAS-4 was administered orally suspended in an aqueous solution of 1% Tween 80 and 0.5 % Carboxy methyl cellulose, q.s., 3 ml/kg, p.o. and 5, 15 and 30 min and 1, 2, 4, and 8 h post dose later blood samples were collected. Whole brain samples were collected at 0.5, 1, 2 and 4 h. Whole blood was collected into chilled EDTA-fortified tubes, centrifuged for 10 min at 5000 rpm (4°C), and stored at -80°C until HPLC analysis. The brain samples were rinsed in phosphate-buffered saline, snap-frozen, and stored at -80°C. Before HPLC analysis, brain samples were thawed to room temperature and subjected to mechanical homogenation by using a IKA homogenizer.

## 2.6. Haloperidol-Induced catalepsy in the rat

The dopamine D2 receptor antagonist, haloperidol (1 mg/kg, s.c.) was administered to induce catalepsy. Thirty minutes after haloperidol administration, rats experience a full cataleptic response. Therefore, at that time point, each rat was placed on a wire mesh screen inclined at 60° with their heads facing up and their forelimbs and hind limbs extended. The time taken for the rat to make a limb movement was measured with a cut-off time of 120 s. Only rats that remained cataleptic for the entire 120 s were used for subsequent drug studies. Test compounds (TAS-4) were administered orally after the 30-min baseline measure, and catalepsy was retested 1, 2 and 4 h after administration. Haloperidol was not readministered.

## 2.7. Potentiation of L-DOPA -induced rotations in 6-OHDA lesioned rats

Animals were anesthetized by intraperitoneal administration

of chloral hydrate (400 mg/kg, i.p.) and treated with desipramine (10 mg/kg, i.p.) 30 min before 6-OHDA injection to block the uptake of the toxin by noradrenergic terminals. Rats were subsequently placed in a stereotaxic frame, the skin over the skull was reflected, and a burr hole was drilled through the skull at the following stereotaxic coordinates: -2.2 posterior from bregma (anterior-posterior) and + 1.5 lateral from bregma (medial-lateral). Subsequently, a total of 8  $\mu$ g of 6-OHDA dissolved in 4  $\mu$ l of saline containing 0.05% ascorbic acid was infused -7.8 mm ventral to the dura at a constant flow rate of 1  $\mu$ l/min using a 48-gauge needle attached to an infusion pump. Two weeks after the lesion, rats were administered apomorphine (0.05 mg/kg, s.c.) and selected on the basis of the number of full contralateral turns during a 2-h testing period (priming test). Rats that made fewer than 100 complete turns were not included in the subsequent studies. To test the effect of the TAS-4 in this assay, TAS-4 (1, 3, 10, 30 and 100 mg/kg, p.o.) was delivered 30 min before the delivery of benserazide (10 mg/kg, i.p.). L-DOPA (4 mg/kg, i.p.) was delivered 30 min later and placed in the rotametry chambers. The number of contralateral rotations was recorded during a 2-h test.

## 2.8. L-DOPA dose finding study

Rats were randomly assigned to two groups. One group received one of five doses of L-DOPA (2, 4, 6, 8 and 10 mg/kg, i.p.) and the second group received the same doses but with an additional administration of 10 mg/kg, p.o. TAS-4 1 h before testing. The number of rotations was recorded in the rotametry chamber for 2 h after L-DOPA administration.

## 2.9. Prevention of L-DOPA sensitization and abnormal involuntary movements

Rats were randomly divided into two groups. One group received a combination treatment of vehicle and L-DOPA (8 mg/kg, i.p.) twice a day for 28 days. The second group received a combination treatment of TAS-4 (3 mg/kg, p.o.) and L-DOPA (4 mg/kg) twice a day for 28 days. Daily treatments were administered in the morning (between 8:00 AM and 10:00 AM) and 12 h later. Rotational behaviour was measured on day 1 (i.e., after the first treatment) and on days 7, 14, 21 and 28 at a fixed time in the morning for each animal.

Turning behaviour was measured by placing rats in Plexiglas hemispherical bowls (50 cm diameter) with sawdust on the bottom. Rats were placed in each apparatus 30 min before compound administration in order to acclimatize and to extinguish any spontaneous turning behaviour. Abnormal involuntary movements were measured visually during turning behavioral tests. According to their topographic distribution abnormal involuntary movements were classified into three subtypes: (1) axial abnormal involuntary movements: torsion of head, neck and trunk towards the side contralateral to the lesion in a still position; (2) limb abnormal involuntary movements: movements of the forelimb and the paw contralateral to the lesion (flexions and extensions of the forelimb and opening and closing of the digits); (3) orolingual abnormal involun-

tary movements: tongue protrusion and jaw movements. Each abnormal involuntary movement was quantified in 1 min testing-period at 15, 30 and 60 min after L-DOPA injection and expressed as total time spent by the rat in each movement.

### 3. RESULTS

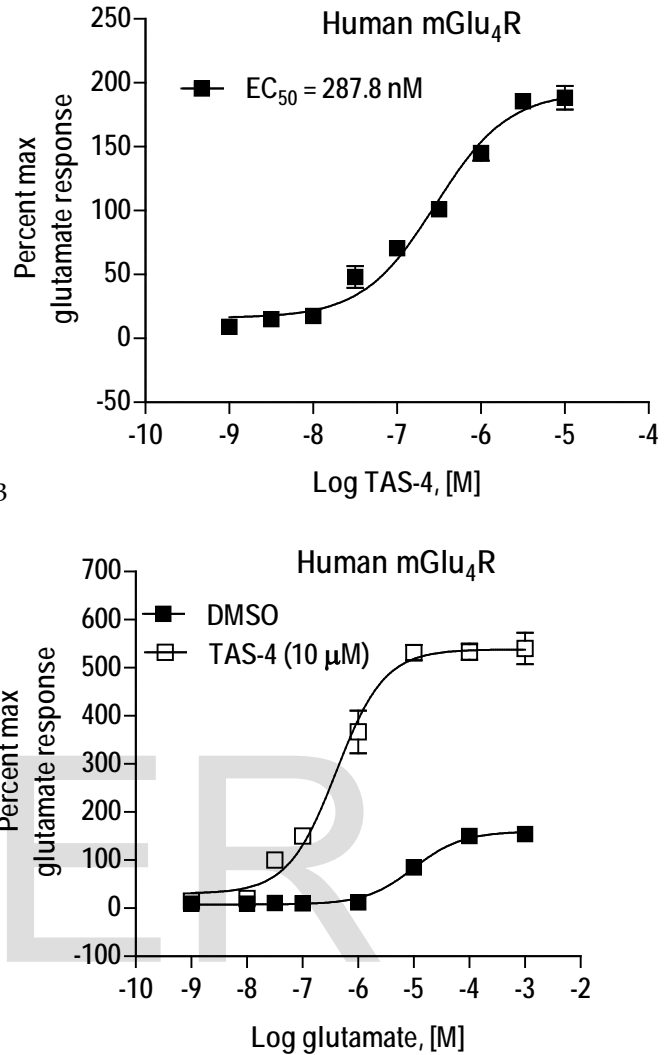
#### 3.1. TAS-4 is a selective positive allosteric modulator of mGluR4

The functional activity of TAS-4 at mGluR4 was measured in a calcium mobilization assay using cells coexpressing human mGluR4 with the chimeric G protein, Gqi5, which links the Gi/o-coupled mGluR4 receptor to the phospholipase C $\beta$ /Ca $^{2+}$ -pathway. TAS-4 produced a concentration-dependent potentiation of the response to an EC $_{20}$  concentration of glutamate with EC $_{50}$  value of 287.8 nM (Fig. 2A). As a test of compound efficacy, the ability of a 10  $\mu$ M concentration of TAS-4 to left-shift the glutamate concentration response curve (CRC) was examined. In these experiments, TAS-4 shifted the glutamate CRC 25 fold to the left (Fig. 2B). Collectively, these in vitro studies suggest that TAS-4 is a potent PAM of multiple signaling pathways that enhances the response of the rat and human mGluR4 receptors to the endogenous agonist glutamate. When tested at a 10  $\mu$ M concentration at each mGluR, TAS-4 exhibited only weak PAM activity (4.3-fold left shift of the glutamate CRC) at mGluR5 (data not shown). When further evaluated in a full concentration-response curve format, TAS-4 exhibited PAM activity at mGluR5 with a potency of 11.2  $\mu$ M (compare with the potency of TAS-4 on the human mGluR4 receptor of 287.8 nM). Taken together, these findings indicate that TAS-4 possesses a relatively clean ancillary pharmacology profile that allows a clear interpretation of the findings from the behavioral studies of mGluR4 activation in vivo.

#### 3.2. In vivo pharmacokinetic profiles of TAS-4

In vivo pharmacokinetic profiles of TAS-4, when Wistar rats received an oral dose of 10 mg/kg of TAS-4, the C $_{max}$  was 1.74  $\mu$ M with a terminal elimination half-life of 1.5 h. Absolute bio-availability was 44 %, showing a good pharmacokinetic profile (Fig. 3). A summary of pharmacokinetic parameters is shown in Table 1. TAS-4 also showed an enhanced central penetration and a total brain-to plasma ratio is approximately 0.6 after oral administration of a 10 mg/kg dose (Table 2).

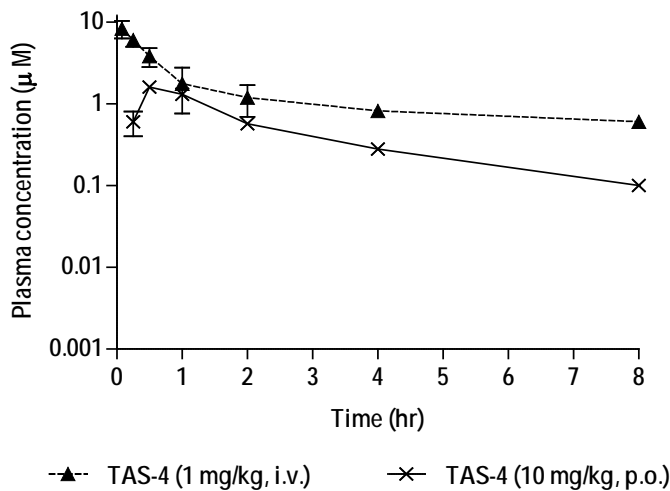
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**Fig. 2.** TAS-4 is a potent and effective PAM of mGlu4R. A and B, TAS-4 exhibits a potency of 287.8 nM at human mGlu4R in the presence of an EC $_{20}$  concentration of glutamate (A) and shifts the glutamate concentration-response curve 25-fold to the left (B). The data represent as Means  $\pm$  SEM. (n = 6 experiments).

#### 3.3. TAS-4 produces a dose-dependent reversal of haloperidol-induced catalepsy

TAS-4 dose-dependently attenuated the cataleptic effects of haloperidol at 1, 2 and 4 h after dosing, with statistically significant effects at doses of 3, 10 and 100 mg/kg at all time points (Fig. 4).



**Fig. 3.** Plasma concentration–time profiles of TAS-4 after i.v. (1 mg/kg) and p.o. (10 mg/kg) administration to male Wistar rats. The data represent as Mean  $\pm$  S.D. (n = 3 animals/route of administration).

**Table 1**  
Pharmacokinetic parameters of TAS-4

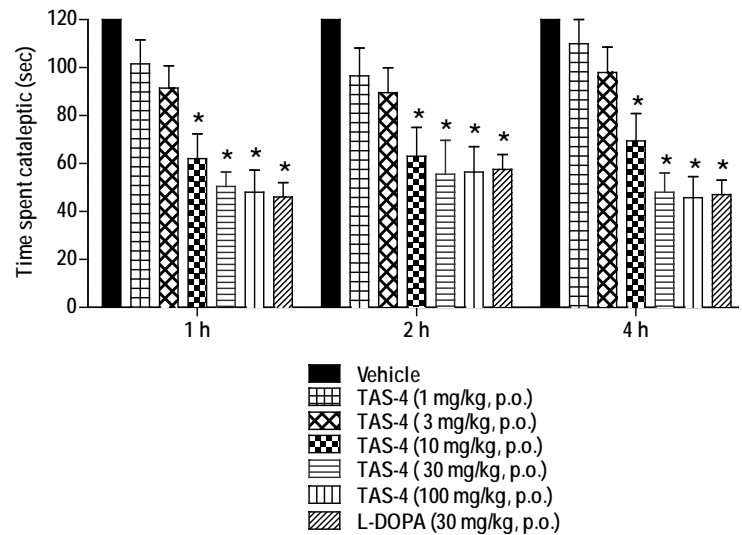
Parameters	I.V. (1 mg/kg)	P.O. (10 mg/kg)
C <sub>max</sub> (µM)	8.29	1.74
T <sub>max</sub> (h)	0.08	0.5
AUC (0-8 h) (µM.h)	5.6	2.6
V <sub>ss</sub> (L/Kg)	1.2	NA
CL (ml/min/Kg)	19.2	NA
T <sub>1/2</sub> (h)	0.9	1.5
F%	NA	44

Pharmacokinetic parameters of NS-1 after i.v. (1 mg/kg) and p.o. (10 mg/kg) administration to male Wistar rats. (Mean, n = 3 animals/route of administration).

**Table 2**  
Plasma and brain concentrations of TAS-4 in Wistar rats

Time (h)	0.5	1	2	4
Plasma (µM)	1.6	1.3	0.57	0.28
Brain (µM)	0.98	0.75	0.34	BQL
Brain/Plasma Ratio	0.61	0.58	0.60	-

Plasma and brain concentrations of TAS-4 after 10 mg/kg, p.o. administration to male Wistar rats. (Mean, n = 6 animals/route of administration). BQL= Below quantification limit.



**Fig. 4.** Effect of TAS-4 (1-100 mg/kg) on haloperidol-induced catalepsy in rats. The data represent as Mean  $\pm$  S.E.M. (n = 8 animals per group). \* p  $\leq$  0.05, One-way ANOVA followed by Dunnett's multiple comparison test as compared with vehicle group at each time point.

### 3.4. Potentiation of L-DOPA -induced rotations in 6-OHDA lesioned rats by TAS-4

The classical method of intracerebral infusion of 6-OHDA involving a massive destruction of nigrostriatal dopaminergic neurons, is largely used to investigate motor and biochemical dysfunctions in Parkinson's disease. Compounds either increases contralateral rotation alone or potentiates the contralateral rotation of sub threshold dose of L-DOPA in this model would be useful for the treatment of Parkinson's disease.

The administration of TAS-4 alone to 6-OHDA-lesioned rats did not result in contralateral rotation behaviour at doses up to 30 mg/kg, p.o. (data not shown). However, TAS-4 (10 mg/kg, p.o.) induced marked contralateral rotation when administered before a subthreshold dose of L-DOPA, 4 mg/kg, i.p. (Fig. 5). The effects were dose-dependent for TAS-4 (1-100 mg/kg, p.o.). TAS-4 at 10, 30 and 100 mg/kg, p.o. showed significant increase in contralateral rotation as compared with L-DOPA (4 mg/kg, i.p.) alone group.

### 3.5. L-DOPA dose finding

TAS-4 (10 mg/kg, p.o.) dosed 60 min before a subthreshold dose of L-DOPA (4 mg/kg, i.p.), elicited a marked contralateral rotation in 6-OHDA rats (Table 3). To establish the dose of L-DOPA that produced the same turning behaviour intensity as the L-DOPA -TAS-4 combination, dose-response studies were performed by administering L-DOPA at 2, 4, 6, 8 and 10 mg/kg, i.p. A dose of 8 mg/kg L-DOPA alone induced a similar number of contralateral turns to that induced by L-DOPA

(4 mg/kg, i.p.) in combination with TAS-4 (10 mg/kg, p.o.). Thus, a dose of 8 mg/kg L-DOPA was chosen for the following experiments.

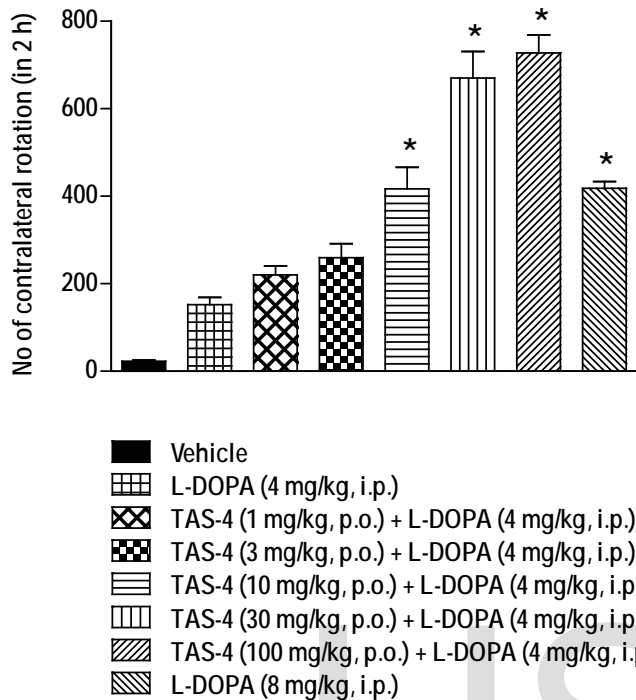
trast, subchronic treatment with TAS-4 (10 mg/kg, p.o.) + L-DOPA (4 mg/kg, i.p.) produced no significant increase in all abnormal involuntary movements during the course of the treatment (Fig. 7A-D).

**Table 3**

Contralateral rotations induced by various doses of L-DOPA in combination with either vehicle or TAS-4.

Treatment Group	Number of Rotations
Vehicle (p.o.) + Vehicle (i.p.)	32 ± 5
Vehicle (p.o.) + L-DOPA (2 mg/kg, i.p.)	45 ± 4
Vehicle (p.o.) + L-DOPA (4 mg/kg, i.p.)	143 ± 13
Vehicle (p.o.) + L-DOPA (6 mg/kg, i.p.)	226 ± 26*
Vehicle (p.o.) + L-DOPA (8 mg/kg, i.p.)	415 ± 69*
Vehicle (p.o.) + L-DOPA (10 mg/kg, i.p.)	604 ± 25*
TAS-4 (10 mg/kg, p.o.) + L-DOPA (4 mg/kg, i.p.)	417 ± 50*

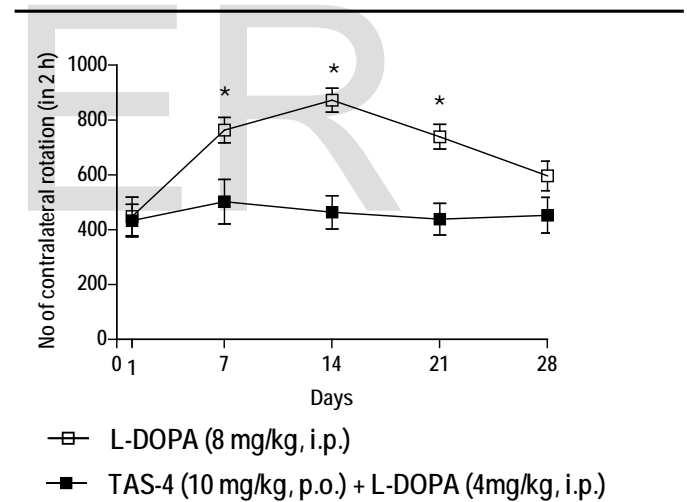
Vehicle or TAS-4 was administered 60 min before L-DOPA, and then contralateral rotation were recorded for 2 h. The data represent as Mean ± S.E.M. (n = 6 animals per group). \* p ≤ 0.05, One-way ANOVA followed by Dunnett's multiple comparison test as compared with Vehicle group.



**Fig. 5.** Potentiation of L-DOPA -induced (4 mg/kg, i.p.) contralateral rotations in 6-OHDA-lesioned rats by TAS-4. The data represent as Mean ± S.E.M. (n = 8 animals per group). \* p ≤ 0.05, One-way ANOVA followed by Dunnett's multiple comparison test as compared with L-DOPA (4 mg/kg, i.p.) group.

### 3.6. Prevention of L-DOPA sensitization and abnormal involuntary movements

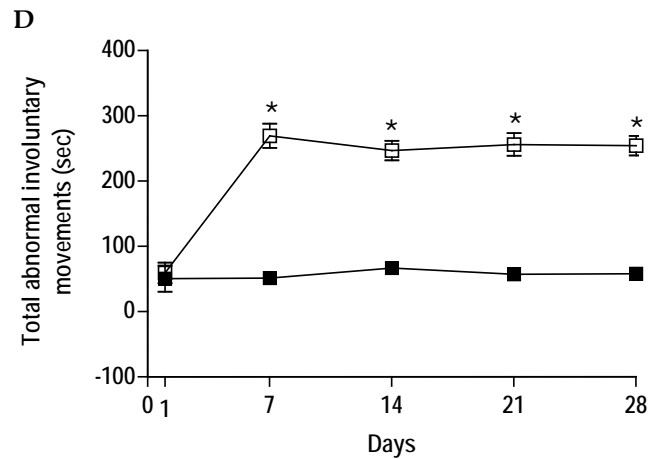
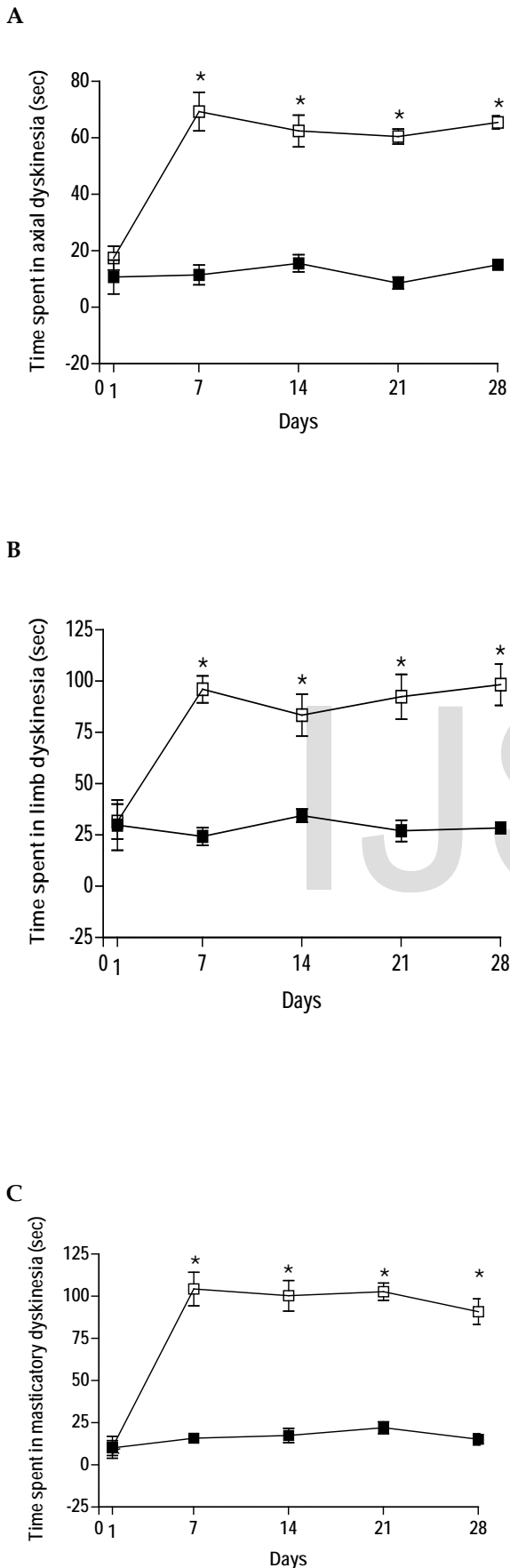
6-OHDA lesioned rats were chronically treated twice a day for 28 days with TAS-4 (10 mg/kg, p.o.) and L-DOPA (4 mg/kg, i.p.) or with vehicle (p.o.) and L-DOPA (8 mg/kg, i.p.). The rotational response to L-DOPA alone gradually increased, reaching a plateau on days 7 to 21 before decreasing by day 28 (Fig. 6). The L-DOPA-induced (8 mg/kg, i.p.) contralateral rotation in this group was significantly different between day 1 and days 7, 14 and 21 (One-way ANOVA followed by Dunnett's multiple comparison test). In contrast, rats receiving TAS-4 (10 mg/kg, p.o.) in combination with L-DOPA (4 mg/kg, i.p.) displayed a rotational response on all subsequent days of the study that was not significantly different from day 1 (Fig. 6). One-way ANOVA followed by Dunnett's multiple comparison test showed a significant difference in the time spent by L-DOPA (8 mg/kg, i.p.) subchronic treated rats in axial, limb, masticatory and total abnormal involuntary movements on days 7, 14, 21 and 28 with respect to day 1 (Fig. 7A-D). By con-



**Fig. 6.** Effect of subchronic L-DOPA (8 mg/kg, i.p.) and TAS-4 (10 mg/kg, p.o.) + L-DOPA (4 mg/kg, i.p.) on contralateral rotation. Time-course analysis revealed a significant difference in the turning behavioural response to subchronic L-DOPA (8 mg/kg, i.p.) from day 1 to days 7, 14 and 21. The data represent Mean ± S.E.M. (n = 8 animals per group). \* p ≤ 0.05, One-way ANOVA followed by Dunnett's multiple comparison test as compared with respective day 1.

## 4. DISCUSSION

Parkinson's disease is a chronic neurodegenerative disorder resulting from the loss of dopaminergic neurons in the



**Fig. 7.** Effect of subchronic L-DOPA (8 mg/kg, i.p.) and TAS-4 (10 mg/kg, p.o.) + L-DOPA (4 mg/kg, i.p.) on abnormal involuntary movements. Time-course analysis revealed a significant difference in the time spent by L-DOPA (8 mg/kg, i.p.) subchronic treated rats in axial (A), Limb (B), Masticatory (C), total (D), abnormal involuntary movements (AIMs) from day 1 to days 7, 14, 21 and 28. The data represent as Mean  $\pm$  S.E.M. (n = 8 animals per group). \*  $p \leq 0.05$ , One-way ANOVA followed by Dunnett's multiple comparison test as compared with respective day 1 data.

substantia nigra (SNc), which provide the major dopaminergic innervation to the striatum and other basal ganglia (BG) nuclei. While dopamine-replacement therapies are useful early in the disease, these treatments generally lose their efficacy as the disease progresses and are associated with numerous dose limiting side effects, including on/off oscillations, dyskinesias, and cognitive impairments [14].

Limitations with current dopamine replacement therapies for the symptomatic treatment of PD have given rise to alternative strategies focused on normalizing synaptic transmission within the indirect pathway of the basal ganglia. Selective mGluR4 PAMs can decrease excessive inhibitory tone at the striatopallidal synapse and reduce motor impairments in animal PD models [15-17]. This approach is attractive for several reasons. First, the relatively restricted distribution of mGluR4 [18,19] suggests that activation of this receptor would produce a somewhat selective decrease in striatopallidal transmission. Second, by targeting the striatopallidal synapse and bypassing the striatal dopamine system, palliative benefit can be achieved while avoiding the severe side effects associated with dopamine-replacement therapy. Finally, by avoiding potential oxidative stress associated with L-DOPA therapy, and by decreasing glutamatergic drive through the indirect pathway onto midbrain dopamine neurons, this approach may theoretically lead to a slowing of disease progression [20]. Unfortunately, early-generation mGluR4 PAMs possessed less selectivity for mGluR4 relative to other mGluRs and unsuitable physio-



chemical properties for systemic dosing to allow broader investigation of the role of mGlu4 in BG circuitry.

VU0364770 compound was active only after subcutaneous administration, less selective to other receptor and less potent in comparison with TAS-4 [12]. ADX88178 is a potent compound as compared with TAS-4 but having a relatively short half-life 0.2 h versus 1.5 h and also did not alter L-DOPA induced dyskinesia. However administration of TAS-4 in combination with sub threshold dose of L-DOPA potentiated the motor effect similar to those induced by a higher dose of L-DOPA without exacerbating the dyskinetic effects [13].

The present pharmacologic characterization of TAS-4 shows that this mGluR4 PAM is an important tool for the modulation of mGluR4 activity in vivo. TAS-4 exhibits nanomolar potency, high selectivity for mGluR4 relative to other mGlu, enhanced physiochemical properties for in vivo dosing, and robust efficacy in rodent PD models when administered in combination with L-DOPA.

TAS-4 potentiated L-DOPA induced contralateral rotations in unilaterally 6-OHDA-lesioned rats. In addition, TAS-4 was effective in reducing catalepsy induced by the D2 receptor antagonist, haloperidol, which is consistent with previous studies [21]. These findings suggest that the compounds are acting, as hypothesized, to provide counterbalance to the loss of D2 receptor-mediated effects on the indirect pathway. Anticataleptic properties have been predictive of clinical efficacy for other antiparkinsonian agents. For example, compounds with potent D2 receptor agonist activity, such as pramipexole, have also been shown to block catalepsy in this assay [22]. These findings collectively support the potential therapeutic potential of mGluR4 PAM for the treatment of Parkinson's disease.

Another important consideration related to these data is the possibility that a selective mGlu4 PAM might induce dyskinesias or potentiate L-DOPA -induced dyskinesias by decreasing GABAergic neurotransmission in the globus pallidus externa [23]. However, although dyskinetic activity would be predicted if all GABAergic neurotransmission was shut down in the globus pallidus externa, mGluR4 PAMs are postulated to have a more subtle modulatory effect on GABAergic signaling. In 6-OHDA-lesioned rats, repeated administration produces behavioral sensitization, manifested as a marked increase in L-DOPA -induced contralateral rotations across days of treatment [24]. Behavioural sensitization has been suggested to predict the development of dyskinesias after chronic treatment with L-DOPA [25].

Here, we found that L-DOPA (8 mg/kg, i.p.) produced behavioural sensitization and abnormal involuntary movements after only 7 days of treatment in 6-OHDA-lesioned rats. When administered subchronically with a threshold dose of L-DOPA (4 mg/kg, i.p.) in combination with TAS-4 (10 mg/kg, p.o.) induced a similar intensity of contralateral rotation to that produced by a full dose of L-DOPA (8 mg/kg, i.p.); however, dur-

ing the course of the treatment this drug combination did not induce sensitization to contralateral turning behaviour and abnormal involuntary movements. Therefore, administration of TAS-4, by potentiating the effect of L-DOPA, induces motor effects similar to those induced by a higher dose of L-DOPA without exacerbating the dyskinetic effects.

In conclusion this study suggest that TAS-4 is potent and selective mGluR4 PAM with robust efficacy in preclinical models of Parkinson's disease when administered in combination with L-DOPA. Our data demonstrate that this highly selective mGluR4 PAM are efficacious in rodent models of Parkinson's disease with a potential for a lack of dyskinesias when coupled with L-DOPA. Furthermore, TAS-4 may treat Parkinson's disease symptoms without inducing dyskinesias when used in combination with a threshold dose L-DOPA . The present findings provide important evidence for the utility of selective mGluR4 PAMs as a novel treatment strategy for the symptomatic treatment of Parkinson's disease and as a possible augmentation strategy with L-DOPA.

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